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A RAPID AND SIMPLE CARBIDE METHOD FOR ESTIMATING MOISTURE IN FLOUR[1]

M. J. BLISH AND B. D. HITES

Department of Agricultural Chemistry, University of Nebraska, Lincoln

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There has been developed in this laboratory a method whereby moisture in flour may be quantitatively determined, within limits of accuracy suitable for most purposes, in approximately five minutes. The procedure involves no new principles, but is merely an adaptation of the familiar reaction between calcium carbide and water, whereby acetylene gas is produced.

The literature records numerous instances in which various applications of this principle have been proposed and used for the determination of moisture in materials of all types. McNeil (1912) suggested a method involving the volumetric measurement of the acetylene produced by the interaction of calcium carbide and the substance under investigation. His work indicated the applicability of the procedure to the estimation of moisture in materials such as flour, soap, leather, powder, vanilla beans, sawdust, butter, and fruit juices.

Many others have reported special methods and applications for the determination of moisture by the use of calcium carbide. No extended review of the literature is attempted here, since this may be obtained by consulting a recent paper by Radeff (1926). In that paper are presented references to previous investigations, and also a report of experiments indicating the usefulness of the method in the analysis of a varied assortment of feeds and food products.

The majority of methods thus far proposed provide for the volumetric measurement of the acetylene, with subsequent reduction of gas volume to standard temperature and pressure. Other proposed methods involve: (1) estimation of weight of acetylene

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by weighing the entire system before and after the formation and escape of the gas, and (2) noting the pressure produced by the gas, using apparatus of constant volume. Korff-Petersen (1913) proposed the latter, and noted the pressure as indicated by a dial pressure-gauge.

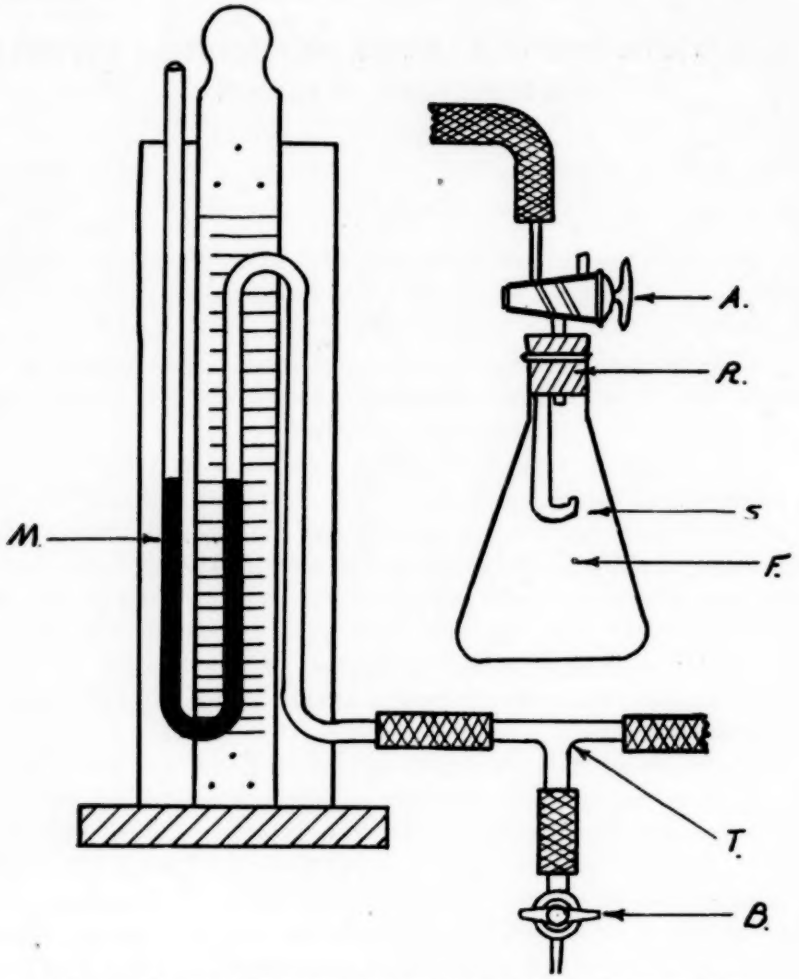

Fig. 1. Diagram of Apparatus Used for Determination of Water in Flour

Experimental

The method that has been successfully applied to wheat flour in this laboratory involves substantially the principle employed by Korff-Petersen (1913). It consists merely of allowing the flour

to react with powdered calcium carbide in a system of constant volume, and by means of a mercury manometer observing the millimeters of pressure developed by the confined acetylene gas.

Apparatus.—The apparatus, which is simple and readily available in any laboratory, is indicated in Figure 1. "F" (see Fig. 1) is a dry Erlenmeyer flask of 300-350 cc. capacity. "R" is a tight-fitting one-holed rubber stopper, into which is inserted, on the upper side, a two-way stopcock "A," by which the flask may be connected either to the manometer "M," or to the outside air. "S" is a bent metal spoon, forced into the lower side of the rubber stopper and projecting down into the flask. The flask is connected to an open mercury manometer "M," by means of pressure tubing. Stop-cock "B" is inserted into the system by means of the small T-tube "T." This serves as a valve which is opened, temporarily, just before the reaction, in order to adjust the pressure of the system to that of the local atmosphere.

Method.—Weigh one gm. flour into the flask, which must, of course, be absolutely dry. Place 2 or 3 gms.[1] *freshly* powdered calcium carbide on the spoon "S." Insert stopper tightly, taking care to avoid spilling any carbide onto the flour. Connect flask with manometer through stop-cock "A." Then open stop-cock "B" for an instant in order to adjust to atmospheric pressure. Close "B," and tip the flask, with shaking, so that the carbide is slowly and gradually spilled upon the flour, and mix thoroughly by vigorously shaking the flask. Repeat the shaking at intervals of one minute, until the difference between the heights of the two mercury columns is constant (usually about 3 or 4 minutes). Record millimeters of difference between heights of mercury columns.

The pressure recorded by the manometer is, of course, proportional to the amount of acetylene gas produced, and is therefore an index to the quantity of water that has reacted with the carbide. However, not all of the flour moisture reacts with the carbide under these conditions, and the amount that does not react appears to be a very definite and sharply defined portion of the total flour moisture. The procedure, therefore, distinguishes distinctly between the portion of the flour moisture that is free or loosely held, and that which is closely bound by the flour particles, to the extent that it does not react with calcium carbide. If the amount of non-reacting water is the same, or very nearly so for all flours under ordinary laboratory conditions, its value may be determined ex-

[1] Need not be weighed, but merely roughly estimated.

perimentally, and applied as a constant by merely adding it to the value for the loosely held water that has reacted with the carbide, as indicated by the manometer. The sum of the two values represents the total flour moisture.

It is first of all necessary to calibrate each apparatus, in order to definitely establish the relation between known quantities of water and observed pressure. This is done by running a few (4 or 5) determinations using varying, known quantities of pure water alone. These values fall on a straight line, when a curve is constructed with weights of water plotted on one axis and units of pressure on the other. Therefore, in order to convert observed units of pressures into corresponding quantities of reacting water, it is necessary merely to refer to the graph. Figure 2 represents the graph for the apparatus used in securing the data herein reported.

The extent to which results with pure water alone are influenced by variations in room temperature is indicated in Fig. 2. The continuous line represents values obtained at a room temperature of 20°C., whereas the broken line indicates results at 28°C. Most room temperatures fall between these limits. Obviously temperature is not a critical factor, although the interests of accuracy are best served by using the curve that corresponds most nearly to the temperature at which the actual determination is made. The curves for 20° and 28° are so close together that values for temperatures in between can be readily interpolated.

In running determinations using water alone, one must take the precaution to add the carbide to the water slowly and gradually, since if added all at once, the sudden and instantaneous evolution of acetylene will be likely to blow out either the stopper from the flask or the mercury from the manometer. In the case of *flours,* within the ordinary ranges of moisture content, however, this precaution is unnecessary.

Experimental

The first step was necessarily to find out whether or not the amount of non-reacting water is sufficiently *constant* for flours of different types and origins to justify further consideration of the method's possibilities. Forty-two flours of varying grades, types, and moisture content were obtained from more than twenty different mills. These samples varied from 0.37 to 1.09% in ash content, and included both hard and soft wheat flours. These flours

were subjected to the carbide procedure as previously described. Duplicate determinations invariably showed extremely close agreement. Quantity of reacting water was in each case ascertained by noting the pressure and consulting the graph. All flours were then analyzed for total moisture, in duplicate, by the alternate official oven method involving exposure to a temperature of 130°C. for one hour. The amount of non-reacting (with carbide) water was in each case calculated by subtracting the quantity of reacting water from the total moisture as determined by the oven method. It was found that for most practical purposes this value may be regarded as constant for different flours.

The next step was to calculate the *average* of all individual values for non-reacting flour moisture, and apply this average as a constant by adding it, in the case of each flour, to the amount of "reacting moisture" as ascertained by the carbide procedure. A comparison of this series of results with the corresponding values obtained by the oven method serves to indicate the degree of reliability to be anticipated when using the proposed carbide procedure alone on flours of unknown moisture content. All experimental data secured from this series of flours are shown in Table I, which follows.

Column 5, Table I, shows for each individual flour the quantity of water that did not react with calcium carbide, as determined experimentally. The actual deviation of each value from 0.0455, the average or mean of all values, is shown in Column 7, in terms of per cent of flour moisture. The data in Column 7 accordingly indicate the extent to which moisture results by the carbide method (involving the constant .0455), when applied to a flour of unknown moisture content, may be expected to approximate those secured by the approved oven method.

Using approved methods of statistical analysis, the standard deviation from the oven method was found to be ± 0.174, in terms of per cent of flour moisture, with ± 0.12 as the probable error of a single determination; and ± 0.0002 (gm) the probable error of the mean (0.0455).

On the whole, the results obtained by the proposed carbide procedure check closely with those secured by the use of the alternate standard oven procedure (1 hour at 130°C.), as is clearly shown by the figures in Column 7, Table I. The largest single deviation is 0.32%, in the case of sample No. 24, where the oven method gave a value of 10.38, while the carbide method showed

TABLE I

CARBIDE METHOD AS APPLIED TO COMMERCIAL FLOURS (1 GRAM SAMPLES)

Sample No.	Total Moisture by Oven Method	Manometric Pressure	"Reacting" Water (From graph)	"Non-reacting" Water (Difference between "Reacting" and Total)	Total Moisture ("Reacting" water plus constant .0455)	Deviation from Oven Method
	Per Cent	Millimeters	Grams	Grams	Per Cent	Per Cent
1	12.57	91.5	.0810	.0447	12.65	+.08
2	13.98	103.00	.0930	.0468	13.85	—.13
3	9.80	55.0	.0520	.0460	9.75	—.05
4	9.57	53.0	.0502	.0457	9.57	.00
5	14.02	103.00	.0930	.0462	13.85	—.17
6	13.65	103.5	.0920	.0445	13.75	+.10
7	9.47	50.5	.0480	.0467	9.35	—.12
8	9.86	55.5	.0522	.0464	9.77	—.09
9	11.92	81.0	.0740	.0452	11.95	+.03
10	12.53	87.0	.0792	.0461	12.47	—.08
11	8.25	39.0	.0380	.0445	8.35	+.10
12	8.76	42.5	.0410	.0466	8.65	—.11
13	8.38	40.5	.0392	.0444	8.47	+.09
14	7.76	36.5	.0340	.0436	7.95	+.19
15	14.29	111.0	.0980	.0449	14.35	+.06
16	14.10	109.5	.0970	.0440	14.25	+.15
17	9.87	55.5	.0504	.0483	9.59	—.28
18	11.30	75.0	.0670	.0460	11.25	—.05
19	11.52	76.0	.0680	.0472	11.35	—.17
20	14.16	106.0	.0940	.0476	13.95	—.21
21	10.22	59.0	.0555	.0477	10.10	—.12
22	10.09	58.0	.0544	.0465	9.99	—.10
23	10.20	57.0	.0538	.0482	9.93	—.27
24	10.38	58.5	.0551	.0487	10.06	—.32
25	8.65	42.5	.0395	.0470	8.50	—.15
26	9.66	55.0	.0500	.0466	9.55	—.11
27	10.16	62.5	.0564	.0452	10.19	—.03
28	9.75	53.0	.0502	.0473	9.57	—.18
29	11.75	82.0	.0725	.0450	11.80	+.05
30	10.30	67.5	.0605	.0430	10.61	+.31
31	10.54	64.5	.0580	.0474	10.35	—.19
32	10.24	59.5	.0540	.0484	9.95	—.29
33	14.72	118.0	.1040	.0432	14.95	+.23
34	14.27	112.0	.0990	.0437	14.45	+.18
35	13.23	94.0	.0836	.0443	12.91	—.32
36	12.83	95.0	.0840	.0443	12.95	+.12
37	11.88	86.0	.0764	.0428	12.19	+.31
38	13.36	101.5	.0900	.0436	13.55	+.19
39	7.17	30.5	.0290	.0427	7.45	+.28
40	7.05	28.5	.0274	.0431	7.29	+.24
41	7.91	38.0	.0358	.0433	8.13	+.22
42	7.91	38.0	.0358	.0433	8.13	+.22
[illegible]	10.995			.0455	10.908	

10.06. This is far better agreement than is ordinarily encountered among collaborative tests upon the same flour sample in different laboratories, with all collaborators using the oven method. The fact that the 42 samples involved in this series represented commercial flours covering a wide range of type, moisture content, and grade, justifies a belief that for almost all practical purposes the carbide procedure, as herein used, is as reliable as the oven method.

It was next considered desirable to conduct a series of determinations on *experimentally milled* flours, exclusively, in order to test the applicability of the procedure to flours of this type. Accordingly, 10 such flours were selected, and the data on these are presented in Table II.

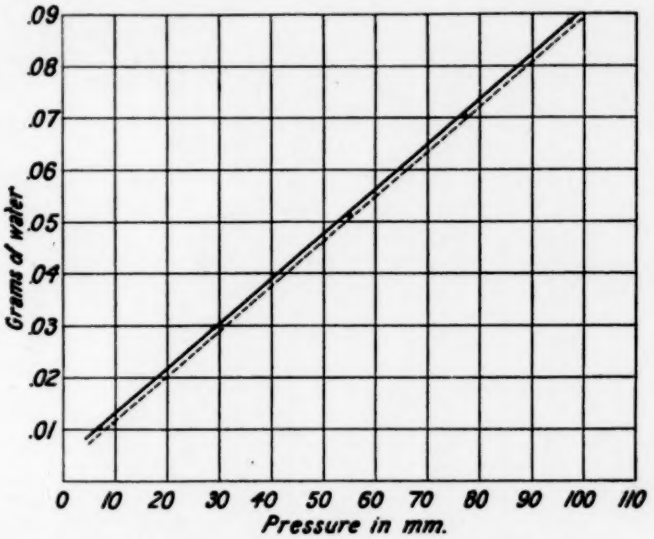

Fig. 2. Graph Showing Relation Between Pressure in Millimeters of Mercury and Grams of Water in the Charge of Flour. Solid Line Represents Values Obtained at 25°C; Broken Line the Values Obtained at 28°C.

Data presented in Table II permit the conclusion that the proposed carbide method is as suitable for experimental as for commercial flours.

Recommended Procedure

Set up apparatus as shown in Figure 1. For routine work it is advisable to have a plentiful supply of flasks of the same volume, so that they may be dried in advance, since with but one flask it is

TABLE II

CARBIDE METHOD WITH EXPERIMENTALLY MILLED FLOURS (1 GRAM SAMPLES)

Sample No.	Total Moisture by Oven Method	Manometric Pressure	"Reacting" Water (From graph)	"Non-reacting" Water (Difference between "Reacting" and Total)	Total Moisture ("Reacting" Water plus constant .0455)	Deviation from Oven Method
	Per Cent	Millimeters	Grams	Grams	Per Cent	Per Cent
1	13.68	102.0	.0901	.0467	13.56	—.12
2	13.54	98.0	.0880	.0474	13.35	—.19
3	13.46	102.0	.0901	.0445	13.56	+.10
4	13.83	105.0	.0946	.0437	14.01	+.18
5	11.66	82.0	.0730	.0436	11.85	+.19
6	11.64	78.0	.0716	.0448	11.71	+.07
7	11.63	78.0	.0716	.0447	11.71	+.08
8	12.97	90.0	.0820	.0477	12.75	—.22
9	13.25	97.0	.0880	.0445	13.35	+.10
10	13.54	99.0	.0890	.0464	13.45	—.09
Average	12.92			.0454	12.93	

necessary to dry it with alcohol and ether after each determination. Standardize the apparatus by making four or five determinations using varying definite amounts of pure water. These quantities of water should range from approximately 0.01 to 0.10 grams, and should be carefully weighed into the flask, the *exact weight* being noted in each instance. Construct graph (as in Figure 2), on which weights of water are plotted on one axis, and the corresponding manometric pressures in millimeters on the other. Subject 1 gram samples of flour to carbide procedure as already described. Consult graph and translate observed millimeters of pressure into quantity of "reacting water." Add the "constant," 0.0455 gm. to this, and the sum is the total weight of flour moisture.

It is obviously possible, and no doubt desirable, to further simplify the procedure by constructing a graph from which *total* flour moisture may be read directly. This is done simply by plotting pressures on one axis, and the sums of the equivalent amounts of reacting water, respectively, and the constant 0.0455, on the other axis. Manifestly, the method is not applicable to flours containing less than 4.5% moisture.

Summary

Total flour moisture may be estimated, with a degree of accuracy sufficient for most purposes, in five minutes, by means of a modified calcium carbide procedure herein described. A one-gram sample of flour is mixed with calcium carbide, (acetylene gas being produced), in a closed system connected with a mercury manometer, and the pressure produced by the acetylene is proportional to the quantity of moisture present. A considerable portion of the flour moisture does not react with carbide under these conditions, but the quantity of "non-reacting" moisture appears to be reasonably constant among flours of different types, grades, and moisture contents.

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WATER IMBIBITION OF FROSTED WHEAT[1]

C. H. Bailey and E. G. Bayfield

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, St. Paul, Minnesota

In the northern portion of the Great Plains area of North America the temperature occasionally falls to a sufficiently low level, just before the spring-sown wheat ripens, to effect a freezing of the immature plant and grain. In most seasons only limited areas are thus affected, and in such instances the freezing temperatures are apt to be encountered at the higher altitudes. Occasionally the spring wheat will be frozen over a large area, as in the instance of the 1928 crop season. Marketing this frosted wheat crop then occasions considerable difficulty, since the relative availability of such wheat for milling and baking purposes is determined, not alone by the percentage of frosted kernels that can be detected, but even more, by the *degree* of damage that has resulted from premature freezing.

The senior author has long held the opinion that the extent to which the individual kernel is thus damaged is determined largely by its relative moisture content at the time it is frozen. Moisture content, in turn, is generally related to the degree of maturity of the plant, and of the grain. That such a relation exists between stage of ripeness, moisture content, and frost damage appears to be established by the careful and detailed work of the chemists of the Montana Agricultural Experiment Station.[2] Thus Johnson and Whitcomb (1927) observed that "when the moisture content at the time of freezing is greater than 44-46 per cent, the frost seriously affects the bread-making properties of the flour."

In general the approach of a moisture level of 40±% in the progressive desiccation of wheat and other cereal kernels signalizes a critical period in the chemical life history of the plant, and of the grain. Translocation of nitrogenous material to the kernel then practically ceases, and protein synthesis ensues. Certain physiologists have suggested that this marked change in chemical properties of the nitrogenous constituents may be the consequence of the concentration of the amino compounds resulting from the loss of moisture. This implies that the production of the more complex protein aggregates from the less complex nitrogenous

[1] Published with the approval of the Director as Paper No. 926, Journal Series, Minnesota Agricultural Experiment Station.

[2] For a bibliography, and review of this and other work on frosted wheat, see A. H. Johnson and W. O. Whitcomb, Mont. Agr. Exp. Sta. Bull. 204 (1927).

"Bausteine" may not occur until a certain concentration of the latter is reached; or at any rate that protein synthesis in such tissues is accelerated by such concentration. Others have maintained that there is evidence of a sort of periodicity in this phase of the life cycle of the plant and its fruit, which may manifest itself by normal protein synthesis even though desiccation is inhibited (see Bailey, 1925).

However this may be, the fact remains that normal ripening is accompanied by progressive desiccation of the grain, and that the appearance of the "finished" proteins and the loss of susceptibility to damage from freezing apparently occur at about the same moisture level, namely 40±%.

It is reasonable to assume that the demonstrable changes in the chemical properties of the proteins and other constituents of the grain which ensue as the moisture content passes from less than 40% to more than 40% may be accompanied by a modification in the colloidal properties of these materials. The latter may in turn be manifested in part by changes in the water-imbibing capacity of these constituents, due to the operation of several factors. So far as the proteins are concerned, the following factors at least deserve consideration: (a) the concentration and nature of certain ions present in the aqueous solution in contact with the proteins; (b) concentration of amino acids, which, as shown by MacDougal (1920) may tend to increase the water imbibition of certain proteins; (c) degree of aggregation, or average molecular weight of the proteins themselves, which may be assumed to increase sharply when the grain approaches ripeness and its moisture content falls below 40%.

Another factor, which may be related to the foregoing in some measure or may be essentially independent, is the gel concentration at the time the processes of ripening are arrested by freezing. Thus Arisz (1915), Bancroft (1921), Gortner and Hoffman (1922, 1927) have indicated that when gelatin gels of varying concentrations were quickly dried, and then placed in contact with liquid water, those gels which originally had the highest concentration of water tended to imbibe the most water on re-wetting. The data presented for gelatin gels by Gortner and Hoffman (1927) are remarkably consistent in their conformity to this rule. The dried gelatin preparations thus reflected their previous history, even when prepared from the same lot of original gelatin.

Description of Materials Used

It thus appeared desirable to attempt to determine the water-imbibing capacity of wheat kernels that had been frozen prematurely and at varying intervals of time before ripening. Through the courtesy of Dr. Arnold H. Johnson and Mr. W. O. Whitcomb of the Montana Agricultural Experiment Station, there were made available to us several excellent series of wheat samples for use in these studies. The histories of, and treatments accorded these samples have been described in Montana Agricultural Experiment Station Bulletin 204. Samples numbered from 1428 to 1447 were of the 1923 crop (see Table I). Heads of Marquis spring wheat were harvested on different dates, one-half of each sampling was dried at once (odd-numbered samples), the other half was immediately frozen at —5°F., then dried and threshed (even-numbered samples). Ten pairs were thus collected, but we were not provided with the non-frozen member of the pair collected on August 9, 1923 (No. 1427).

Another like series of samples was collected in 1926 and numbered from 2872 to 2887 (see Table II).

A third series, numbered 2662 to 2672, was harvested from wheat sown at different intervals of time, the seeding dates being shown in Table III. On September 22 a minimum temperature of 28°F. was recorded, followed by a minimum of 18°F. on the 23rd. Samples designated "a" were at once harvested and dried. In the instance of 4 plots a portion of the plants was allowed to stand in the field for 18 days, then harvested and dried, and the resulting wheat samples are designated as "b."

Experimental Methods

After some preliminary experiments, a purely empirical procedure was evolved and followed in all of our experiments. A sufficient number of entire kernels from each lot to equal 2± grams were weighed on the analytical balance. As will appear later, the exact weight of this charge was not important. These samples were then submerged in cool distilled water and placed in a refrigerator, maintained at 4±°C., for 72 hours. The temperatures at which grain and water are maintained may have had some bearing upon the quantity of water absorbed in 72 hours, since Brown and Worley (1912) have shown that barley kernels absorbed water more rapidly when the temperature was elevated.

At 4°C. no mold growth was evident during the period of treatment nor was there any perceptible growth of the radical and it was assumed that enzymic activity would be at a low level, although the imbibition of water proceeded readily. The kernels were then removed from the water, drained momentarily, and their surface dried by rolling them about between dry blotting paper until they exhibited no tendency to adhere to each other. The kernels were then placed in a tared aluminum drying dish, such as is used in ordinary moisture determinations, the dish covered, and weighed on an analytical balance. The dish, with cover removed, was then placed in the chamber of a vacuum oven and dried at a high vacuum and a temperature of 96°C. for 24 hours. While they may not have reached constant weight during this period, preliminary experiments indicated that the subsequent loss in weight was of small magnitude. Moreover the whole treatment in drying must be regarded as empirical, since the experience in these laboratories has previously indicated that the moisture content of entire or unground cereal grains cannot be determined by direct drying in vacuo. At least, when so determined the results usually are lower, in terms of per cent, than when the ground kernels are analyzed, even when the grinding is conducted under conditions which might be assumed to occasion a loss, rather than a gain, in moisture content. A more refined procedure for determining the percentage of moisture in these water-soaked kernels might appear to be desirable, but the difficulties of grinding them, without loss of moisture through evaporation, will be at once apparent to those who have had experience in dealing with such material. In these early studies a more refined technique than that described as employed by us did not appear to be justified.

From the weight of the dried kernels, the percentage of total imbibed moisture present on the "dry basis" could readily be calculated. This represented the centigrams of moisture imbibed by each 100 centigrams of "dry" substance. These values were also converted into terms of the percentage of moisture on the wet basis, present in the soaked grains. This represented the number of centigrams of moisture included in each 100 centigrams of the soaked grains at the time the latter were removed from the water after 72 hours of immersion. Both sets of calculations are included in the results recorded in Tables I, II, and III.

Results of Observations

In Table I are recorded the results of observations upon the Marquis wheat samples of the crop season of 1923. These were harvested in the head on the dates shown, the samples marked "F" being frozen at —5°F., then thawed, and at once dried. The other samples were not frozen, but were dried in the heads at once, and then threshed for analysis. The percentage of moisture in the kernels when harvested, and the amino-nitrogen content were determined and reported by Johnson and Whitcomb (1927).

It appears that freezing these heads arrested the process of converting the simpler nitrogen compounds into proteins, as shown by the higher amino-nitrogen content of the frozen samples in comparison with the corresponding non-frozen grain harvested on the same date. In the instance of samples harvested on August 17 and August 21, respectively, the results of baking tests recorded in Table 9 of Montana Agricultural Experiment Station Bulletin 204 indicate a substantial difference in baking quality in favor of the non-frosted sample in each pair. In the three succeeding pairs of samples there was no evidence of a difference in baking strength in favor of the non-frozen material. If our interpretation of the Montana data is correct, we would assume that beginning with the samples harvested August 31, and thereafter, the wheat was essentially "sound" so far as can be deduced from the results of chemical analysis, and of milling and baking tests. Moreover, that represents the period when the moisture content of kernels in the head was reduced to less than 40%.

In the light of our later determinations of water-imbibing capacity of these same samples, and confining comparisons to the frozen samples alone, we find that when the percentage of imbibed moisture on the dry basis was equivalent to less than 70%, baking quality of the flour was seemingly normal. As baking quality departed from the normal in the instance of samples harvested August 25 and earlier, the percentage of imbibed water likewise increased.

When amino-nitrogen content is used as the criterion of frost damage, a definite relation between the percentage of this constituent and water imbibition of the kernels can be observed. The coefficient of correlation of these two series of data was accordingly computed and found to be $r = 0.93 \pm 0.03$. This affords a mathematical confirmation of the general assumption that the degree of aggregation of the nitrogenous constituents of the grain may be related to their water-imbibing capacity.

TABLE I

WATER IMBIBITION OF ARTIFICIALLY FROZEN MARQUIS SPRING WHEAT CROP OF 1923

Sample No.	Date Collected*	Treatment* F = frosted N = non-frosted	Amino Nitrogen in Wheat*	Moisture Content at Time of freezing*	Water Imbibed	
					Wet Basis	Dry Basis
			% of total N	%	%	%
1428	Aug. 9	F	14.0	69.4	66.16	195.53
1429	Aug. 13	N	1.0	62.5	48.31	93.46
1430	Aug. 13	F	13.6	62.5	57.15	133.36
1431	Aug. 17	N	1.1	56.2	43.53	77.10
1432	Aug. 17	F	7.8	56.2	53.00	112.78
1433	Aug. 21	N	0.8	50.6	39.01	63.97
1434	Aug. 21	F	4.2	50.6	45.23	82.59
1435	Aug. 23	N	0.7	46.5	40.44	67.90
1436	Aug. 23	F	2.6	46.5	42.40	73.61
1437	Aug. 25	N	0.8	46.5	38.67	63.06
1438	Aug. 25	F	2.4	46.5	42.12	72.77
1439	Aug. 29	N	0.7	43.5	39.36	64.92
1440	Aug. 29	F	1.9	43.5	39.31	64.77
1441	Aug. 31	N	0.8	38.7	40.06	66.85
1442	Aug. 31	F	2.0	38.7	40.09	66.93
1443	Sept. 3	N	0.8	34.1	38.96	63.83
1444	Sept. 3	F	1.4	34.1	39.67	65.76
1445	Sept. 6	N	0.8	....	38.42	62.39
1446	Sept. 6	F	1.2	....	39.40	65.01
1447	Sept. 18	N	0.7	9.3	38.88	63.62

* Reported in Mont. Agr. Exp. Sta. Bull. 204, pages 52 and 63.

Data resulting from our observations upon the 1926 crop samples that were artificially frozen are recorded in Table II. A comparison of our findings with the data reported by Johnson and Whitcomb (1927) leads to conclusions essentially similar to those based upon the 1923 samples (Table I). Samples harvested on August 26, when the moisture content was 42%, and subsequently frozen, gave baking tests equal to those reported for the non-frozen, and presumably normally ripened sample harvested August 31. In fact, the same might be said of the frozen sample of August 23, when the moisture content was 44.4%, although the latter contained more than the percentage of amino nitrogen which would ordinarily be regarded as the safe limit for sound grain. The yield of flour and percentage of germination of this sample (No. 2881) were also appreciably below that of normal grain. We are accordingly inclined to regard the material harvested August 26 as the earliest of the normal or sound frosted wheat collections. If this assumption be correct, then we again have an indication that

the approach to an imbibition of 70% of water (dry basis) marks the level at which the damage by frost may be appreciable.

The percentages of amino nitrogen reported in this series of samples are more erratic in their relation to the history of the material than in the instance of the 1923 crop samples. Despite this fact, the coefficient of correlation of amino nitrogen with water imbibed was found to be $r = 0.59 \pm 0.11$, which is a significant correlation.

TABLE II

WATER IMBIBITION OF ARTIFICIALLY FROZEN MARQUIS SPRING WHEAT CROP OF 1926.

Sample No.	Date Collected*	Treatment* F=frosted N=non-frosted	Amino Nitrogen in Wheat*	Moisture Content at Time of freezing*	Water Imbibed	
					Wet Basis	Dry Basis
			% of total N	%	%	%
2872	Aug. 11	N	1.25	54.95	43.87	78.16
2873	Aug. 11	F	4.37	54.95	46.51	86.97
2874	Aug. 14	N	1.24	53.75	44.10	78.88
2875	Aug. 14	F	6.42	53.75	42.02	72.50
2876	Aug. 17	N	1.25	49.45	40.11	66.98
2877	Aug. 17	F	2.78	49.45	41.96	72.30
2878	Aug. 20	N	1.22	46.22	39.78	66.05
2879	Aug. 20	F	5.17	46.22	44.29	79.50
2880	Aug. 23	N	1.02	44.39	39.20	64.47
2881	Aug. 23	F	3.18	44.39	41.32	70.42
2882	Aug. 26	N	0.76	41.98	38.85	63.53
2883	Aug. 26	F	2.37	41.98	40.52	68.13
2884	Aug. 28	N	0.70	38.70	38.97	63.86
2885	Aug. 28	F	1.44	38.70	39.86	66.28
2886	Aug. 31	N	0.80	34.27	39.22	64.54
2887	Aug. 31	F	1.32	34.27	38.87	63.59

* Reported in Mont. Agr. Exp. Sta. Bull. 204, pages 56 and 65.

Wheat Frozen in Field

In 1926 the wheat plots at the Montana station were planted at different dates. When frozen in the field on September 22, and again on September 23, they accordingly represented grain at different stages of maturity. Plots planted on May 15, 22, and 29, respectively, were evidently mature before September 22, since the description of the wheat and its behavior in baking indicate that samples 2670, 2671, and 2672 were normal and essentially free from damage. Sample 2668b, which contained 37.5% of moisture when frozen, was reported as 84% frost damaged, and only 50% of the kernels germinated. Color and texture of bread were also somewhat impaired. This appears to represent one of the abnor-

mal cases where greater damage occurred than might be anticipated from the moisture content of the grain at the time it was frozen.

The relation of water imbibition to the history and properties of these wheat samples is evident from the data recorded in Table III. In general the degree of immaturity of the wheat at the time of freezing is reflected in the percentage of imbibed water. Wheat kernels which imbibed more than 70% of water (dry basis) were apparently definitely damaged, with the border-line samples approaching 70%.

TABLE III

WATER IMBIBITION OF MARQUIS SPRING WHEAT PLANTED ON DIFFERENT DATES AND SUBSEQUENTLY FROZEN IN THE FIELD. CROP OF 1926.

Sample No.	Date Planted*	Amino Nitrogen in Flour*	Moisture Content at Time of freezing*	Water Imbibed	
				Wet Basis	Dry Basis
		% of total N	%	%	%
2672	May 15	0.57	.....	37.35	59.61
2671	May 22	0.61	.....	37.19	59.22
2670	May 29	0.55	.....	37.59	60.24
2668b	June 5	2.80	37.50	38.65	63.00
2668a	June 5	2.94	37.50	39.79	66.09
2666b	June 12	4.25	48.55	44.20	79.23
2666a	June 12	4.23	48.55	45.48	83.42
2664b	June 19	9.25	61.51	60.73	154.68
2664a	June 19	9.14	61.51	57.46	135.10
2662b	June 26	14.41	68.40	67.80	210.54
2662a	June 26	11.02	68.40	69.00	222.54

* Reported in Mont. Agr. Exp. Sta. Bull. 204, pages 55 and 64.

Using the percentages of amino nitrogen as the chemical criteria of the extent of frost damage, the coefficient of correlation of these data, and the percentage of imbibed water was computed, and $r = 0.95 \pm 0.02$, which constitutes almost a perfect correlation.

Summary

Water-imbibing capacity of entire wheat kernels was found to be related to their previous history. Those grains which were frozen when their moisture content was greater than 40% would imbibe more water than the normal or mature kernels. There was a high correlation between the amino nitrogen content of such wheat and the percentage of imbibed water. A survey of the data further indicates that the level of water content, on wetting the wheat, tended to return to that of the grain at the time it was

frozen, when the latter was in excess of 40%. Thus, the water-imbibing capacity of wheat appears to afford an approximate measure of the extent of damage effected by premature freezing.

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WHEAT AND FLOUR STUDIES XVI. THE EFFECT OF YEAST FERMENTATION ON THE VISCOSITY OF FLOUR-WATER SUSPENSIONS[1]

ARNOLD H. JOHNSON AND JESSE GREEN
Agricultural Experiment Station, Bozeman, Montana

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The effect of added yeast on the rate of proteolysis in flour-water suspensions and in bread doughs has been the subject of a large amount of research. As Olsen and Bailey (1925) have made an excellent survey of the literature bearing on the proteoclastic activity of yeast and the mechanism of action of the yeast proteases, this need not be repeated here. As to whether normal baker's yeast possesses proteoclastic activity they state that "a review of the literature does not afford a basis for any definite conclusion." From their own work they conclude that the proteases contributed by sound, normal, intact yeast cells (baker's yeast) are negligible in their effect upon the properties of gluten during a 4 or 5 hour fermentation period. The data from which they drew their conclusions were obtained from a study of the effect of yeast fermentation on the viscosity of flour-water suspensions, the flour-water ratio being about 1 to 4. Although a reduction in viscosity of the flour suspension occurred during autolysis with yeast, they attributed this not to yeast proteases but to acids elaborated by the yeast during the fermentation of the flour suspension. When dilute alkali was periodically added to the fermenting flour suspension to prevent changes in H-ion concentration the extent of the change in viscosity was slight. Also flour suspensions rendered acid artificially with lactic acid exhibited decreases in viscosity comparable with those of similar suspensions rendered acid to the same degree by the action of yeast.

Sharp and Gortner (1924) also measured the changes in the imbibitional properties of the wheat flour proteins which were brought about by fermentation in normal bread doughs. They found that the viscosity of dough suspensions extracted with distilled water increased to a maximum as fermentation progressed. The suspensions used by Sharp and Gortner were prepared from normal bread doughs containing salt, sugar, and lard, and were obtained by suspending portions of the dough in 500 cc. distilled

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water and then extracting with 3 additional 500 cc. portions of distilled water.

As indicated by Sharp and Schreiner (1926) the procedure followed by Olsen and Bailey differed in several important particulars from that of Sharp and Gortner. Olsen and Bailey used a simple flour-in-water suspension with a flour-water ratio of about 1 to 4. while Sharp and Gortner used a bread dough with a flour-water ratio of 1 to 0.6, to which also were added salt, sugar, and lard. Sharp and Gortner removed the greater part of the extracted electrolytes before acidulating with lactic acid, while Olsen and Bailey used unextracted suspensions. These differences in method of procedure may help to explain the apparent disagreement in the results.

In the meantime Johnson and Bailey (1924) had found that the viscosity of suspensions of cracker dough increased slightly during early stages of fermentation but thereafter decreased very markedly. This observation was more in accordance with the work of Olsen and Bailey in spite of the fact that the method of procedure was approximately that of Sharp and Gortner, the flour to water ratio being about 1 to 0.3. It should be mentioned, however, that fermenting cracker dough does not contain added salt and sugar, while bread dough contains these two constituents. As our knowledge concerning the factors affecting the viscosity determination is much more complete now than at the time the preceding work was done, it appeared worth while to reinvestigate the problem with the purpose of determining the changes in imbibitional property of the flour proteins during fermentation and perhaps to correlate these changes with the proteoclastic activity contributed by yeast to flour suspensions or flour doughs.

Before going on to the experimental part it might be well to mention some of the recent work on proteoclastic activity in fermenting media, in cases in which methods other than the viscometric method have been used for its measurement. Johnson and Bailey (1924) showed that a marked increase occurred in the protein non-precipitable with copper and tin salts during the long fermentation of cracker doughs. Cairns and Bailey (1928) found that fermenting flour-water suspensions increased in their amino-nitrogen content as determined by the formol titration even though the time of fermentation was as short as 5 hours. The data of these investigators indicates therefore that some proteolysis of flour protein occurs during fermentation with yeast. Olsen and Bailey

(1925) on the other hand found that after 4 hours fermentation, flour-water suspensions showed no substantial increase in the quantities of nitrogen not precipitable by the copper and tin reagents, and the data of Sharp and Schreiner (1926) actually show a decrease in the amino-nitrogen content of dough suspensions during fermentation. Sharp and Schreiner state further that baker's yeast produces no changes in the protein constituents of flour so far as the method of solubility analysis used by them would indicate. From the results cited it appears therefore that there is much conflicting data as to the changes undergone by the flour proteins during the fermentation of flour suspensions and flour doughs with yeast. As has been stated it is now our purpose to repeat some of the work which has been done using chiefly the viscometric method of Johnson, Herrington, and Scott (1929).

Experimental

In order to determine the effect of acid alone on the imbibitional capacity of the flour proteins, 20-gram portions of flour were suspended in water and the suspensions acidulated with varying quantities of 0.1N HCl to give the H-ion concentrations shown in Table I. A patent flour of 12.37% protein and 0.40% ash was used for all the work reported in this paper. This flour had aged for some time, as indicated by the relatively high H-ion concentration of the original flour. The flour suspensions were incubated at 25° and 40°C. for 1 and 4 hours at the end of which times their volumes were made to 100 cc. The suspension was then poured into the cup of the MacMichael viscometer and after acidulation with 2 cc. of syrupy lactic acid the viscosity was determined in the usual way using a No. 30 wire. The results obtained are given in Table I. These results indicate that acid alone is able to effect a diminution in viscosity. The greatest decreases in viscosity occurred when suspensions of high H-ion concentration were digested for 4 hours at 40°C. Under these conditions of digestion as shown by the data in Table I the viscosity decreased from 52° MacMichael for the original suspension digested without added acid to 20° MacMichael for the suspension digested with 10 cc. of 0.1N HCl, or sufficient to give the suspension an H-ion concentration equivalent to pH 4.11.

Johnson and Herrington (1928) noted that flour-water suspensions which had been acidulated to approximately pH 5 and extracted according to the method of Gortner (1924) exhibited great-

TABLE I

VISCOSITIES OF FLOUR-WATER SUSPENSIONS OF DIFFERENT H-ION CONCENTRATIONS DIGESTED AT 25° AND 40° C FOR 1 HOUR, AND FOR 4 HOURS

N/10 HCl Added cc.	pH	Viscosity when digested at 25°C		Viscosity when digested at 40°C	
		1 Hour	4 Hours	1 Hour	4 Hours
		Degrees MacMichael		Degrees MacMichael	
0	5.61	50	54	59	52
1	5.41	49	56	59	50
2	5.19	48	53	56	42
3	4.95	46	53	57	39
5	4.61	45	50	56	32
7	4.36	47	47	55	24
10	4.11	44	46	56	20
15	3.83	43	41	55	16
20	3.67	41	39	54	15
30	3.45	42	41	55	16
40	3.30	41	34	53	15
50	3.15	40	39	52	17
60	3.07	43	37	50	18
70	2.99	40	32	48	20

er viscosities than flour-water suspensions extracted without acidulation. As seen from Table I the greatest changes in viscosity occur at higher H-ion concentrations than that equivalent to pH 5. It therefore seemed desirable to determine whether a decrease in viscosity could also be induced in extracted suspensions when the H-ion concentration was greater than that equivalent to pH 5. Two flour-water suspensions containing 30 cc. of 0.1N HCl were therefore digested for 4 hours at 40°C. A third suspension not containing the added acid was also digested under comparable conditions. At the end of the digestion period sufficient standard alkali was added to neutralize the acid in one of the suspensions containing the added acid. Five grams of NaCl was also added to this suspension as well as to the check suspension containing no added acid. This large quantity of salt was used in order to mask any effect which the small quantity of NaCl formed by the neutralization of the acid might have on the efficiency of the subsequent removal of electrolytes. These three suspensions were then extracted three times with 1000 cc. of distilled water, the first time at 40° and the last two times at 25°C. The residue finally obtained was made up to 100 cc., poured into the cup of the viscometer, and after acidulation with 0.5 cc. of 20 per cent lactic acid the viscosity determined in the usual way. The results obtained are given in Table II. The data indicate that in these extracted suspensions the viscosity also decreased when the digestion was conducted at H-ion concentrations as high as that equivalent to pH 3.5 even when the acid added was completely neutralized before the extraction procedure. More protein was decanted in the case of the neu-

TABLE II

VISCOSITY OF EXTRACTED FLOUR-WATER SUSPENSIONS DIGESTED FOR 4 HOURS AT PH 5.68 AND AT PH 3.55 BEFORE EXTRACTION

Treatment	pH of 1st 1000 cc. Decantate	Viscosity	Total Protein Decanted	
		degrees MacM	cgm.	%
Digested 4 hours, then 5 gm. NaCl added	5.68	194	18.3	46.8
Digested 4 hours with 30 cc. of 0.1N HCl, then 30 cc. of 0.1N NaOH plus 5 gm. NaCl added	5.65	143	21.9	56.0
Digested 4 hours with 30 cc. 0.1N HCl	3.55	79	29.2	74.7

tralized suspension than in the case of the suspension containing no added acid, but it is not believed that this was responsible for its lower viscosity, as the data of Johnson and Herrington (1928) indicate that for patent flours, suspensions of maximum viscosity result when between 50% and 60% of the protein is removed by decantation. A part of the larger decrease in viscosity of the non-neutralized acidified suspension may be due to removal of some of the protein responsible for increased viscosity, but again a part of the decrease is undoubtedly due to the effect of the acid conditions of the digestion. From these experiments it appears, therefore, that in order to determine the effects of yeast alone on the viscosity of flour-water suspensions, the H-ion concentration of the fermenting media must be rigidly controlled.

It was first attempted to keep the H-ion concentration constant during fermentation by allowing the fermentation to proceed in strongly buffered solutions. A liter of solution containing 32 gm. of KH_2PO_4 and 50 gm. of $Na_2HPO_4 \cdot 12H_2O$ was therefore prepared. This buffer solution gave an H-ion concentration equivalent to pH 6.12. Suspensions were prepared using 75 cc. of this solution and 18 gm. portions of flour. The equivalent of 2 gm. of yeast was then added and the preparation allowed to ferment. The temperature of fermentation was 30°C. The yeast used in the experiment was thoroughly washed, 20 gm. of yeast being washed 5 times with 250 cc. of distilled water and the washed residue made up to 250 cc., 25 cc. being used for each fermentation test. At the end of the fermentation period, the fermenting mixture was made up to 1,000 cc. and the extraction and viscosity determination conducted exactly according to the procedure of Johnson, Herrington, and Scott (1929). It was found, however, that when such a mixture was fermented for even as long a period as 24 hours a decrease in viscosity

did not result but instead a slight increase. It was thought that by making the buffer more acid, changes in viscosity might occur during fermentation. Concentrated acetic acid was added to 2 portions of the original buffer solutions so that other buffers of pH values 5.41 and 5.22 were obtained. Eighteen gram portions of flour were then suspended in 75 cc. of the buffers, these suspensions having pH values of 6.12, 5.41, and 5.22. Pure distilled water was used in the preparation of one pair of suspensions having H-ion concentrations equivalent to pH 5.64. Twenty-five cc. of yeast suspension prepared as already described was added to one set of the suspensions, and 25 cc. of distilled water was added to the other set. The flasks were then set in a water bath maintained at 30°C. and fermentation allowed to proceed. At the end of one and nine hours the suspensions were removed from the water bath and after washing with three 1,000 cc. portions of water according to the procedure of Johnson, Herrington, and Scott (1929) the viscosities were determined in the usual way. The results obtained are given in Table III.

The data in Table III show that in the presence of these buffer solutions the yeast did not operate to decrease the viscosity of the extracted acidulated suspension. On the other hand the suspensions fermented with yeast for 9 hours had slightly higher viscosities than the corresponding suspension not containing yeast. Also the greater the H-ion concentration of the buffer solution the higher the final viscosity. It appears, therefore, that the phosphate salts stabilize the flour protein, making its imbibitional capacity less susceptible to decrease due to action of acid and yeast. The unbuffered suspension fermented with yeast showed a decrease in viscosity to 66° and 0° MacMichael during 1 and 9 hours respectively. As 95.7 per cent of the protein was lost in the case of the suspension fermented for 9 hours, the decrease in viscosity could be due to this fact alone. In the case of the suspension fermented for 1 hour, however, the protein decanted was less than that decanted from the flour plus buffer of pH 5.22 plus yeast suspension, and since the viscosity decreased to 66 in the first case and increased to 234 in the last, the decrease in viscosity can hardly be attributed entirely to loss of protein resulting from decantation.

As the presence of phosphate salts appeared to inhibit the effect of acid and of yeast fermentation on the water-imbibing capacity of the flour proteins, it appeared desirable before proceeding further to investigate the effect on this phenomenon of NaCl com-

monly used in breadmaking. The experimental procedure was the same as before, 4.5% NaCl solutions being used instead of the buffer solutions. The data in Table III show that not only did the presence of NaCl in the fermenting suspension prevent a decrease in the imbibitional capacity but it actually effected a marked increase in this property. Extraction with NaCl alone yielded a higher viscosity than did extraction with distilled water. Before interpreting this observation, however, further work will be necessary. The much greater increase in the viscosity of the fermented suspension can probably be attributed to the more acid conditions of its extraction together with the protective action of the salt. The viscosity in this case was 454° MacMichael in spite of the fact that 60.7% of the total protein had been extracted. The viscosity of the check suspension digested with neither acid nor salt was 149° MacMichael, 45.8% of the protein being extracted. It should perhaps be stated that the presence of salts used in these experiments did not appear to interfere with fermentation as in many cases the stoppers were explosively forced from the bottles in which fermentation was going on.

In order to show that the large decrease in viscosity occurring in suspensions allowed to ferment without added salts was not due entirely to loss of most of the protein, several experiments were conducted in which the loss of protein was prevented. Suspensions without added salt or buffer were allowed to ferment for 9 hours, at the end of which time 4.5 gm. of salt was added to one, 20 cc. of 0.1N NaOH to another, and 4.5 gm. of salt plus 20 cc. of 0.1N NaOH to a third. The data in Table III show that excessive loss of protein was prevented in this way, yet the viscosities decreased to 15, 11, and 13 respectively. It appears, therefore, that yeast fermentation has effected an irreversible change in the water-imbibing capacity of the flour proteins.

As the presence of salts had so marked an influence on the effect of yeast fermentation on the rate of decrease of viscosity of flour-water suspensions, buffer solutions could not be used for controlling the H-ion concentration. In order to keep the H-ion concentration constant during fermentation it was therefore necessary to neutralize the acids of fermentation at frequent intervals as Olsen and Bailey (1925) had done. Experiments were accordingly conducted at several pH values by adding dilute alkali to the original suspension before the addition of the yeast. In these experiments the method of procedure was slightly different. Twenty

TABLE III

EFFECT OF PRESENCE OF PHOSPHATE BUFFER AND NaCl ON VISCOSITY OF FLOUR-WATER SUSPENSIONS FERMENTED WITH YEAST

Treatment	1 Hour After Preparation				9 Hours After Preparation			
	pH	Viscosity	Total protein decanted		pH	Viscosity	Total protein decanted	
		degrees MacM	cgm.	%		degrees MacM	cgm.	%
Flour plus water	5.64	154	14.35	36.7	5.64	149	17.90	45.8
Flour plus water plus yeast	5.27	65	21.40	54.8	4.00	0	37.40	95.7
Flour plus buffer No. 1	6.12	99	13.10	33.5	6.12	101	14.70	37.6
Flour plus buffer No. 1 plus yeast	6.12	110	16.00	41.0	6.02	112	15.50	40.0
Flour plus buffer No. 2	5.41	165	13.95	35.7	5.41	179	15.80	40.5
Flour plus buffer No. 2 plus yeast	5.41	176	17.20	44.0	5.35	186	17.70	45.3
Flour plus buffer No. 3	5.22	179	17.10	43.8	5.22	224	19.90	50.9
Flour plus buffer No. 3 plus yeast	5.22	177	19.85	50.8	5.22	234	22.10	56.6
Flour plus NaCl	5.68	254	19.00	48.6	5.68	289	20.20	51.7
Flour plus NaCl plus yeast	5.17	249	22.30	57.1	4.93	454	23.70	60.7
Flour plus water plus yeast, fermented 9 hrs. then 4.5 gm. NaCl added					5.00	15	28.60	70.6
Flour plus water plus yeast, fermented for 9 hrs., then 20 cc. N/10 NaOH added					6.28	11	21.00	53.8
Flour plus water plus yeast, fermented 9 hrs., then 20 cc. N/10 NaOH plus 4.5 gm. NaCl added					6.32	13	19.20	49.2

gram portions of flour were suspended in 900 cc. of distilled water and the proper quantity of alkali added to give the desired H-ion concentration. The suspension containing no added alkali had pH 5.65; that containing 3 cc. of N/10 NaOH, pH 6.10; that containing 6 cc. of alkali, pH 6.70; and that containing 10 cc. of alkali, pH 7.25. Twenty-five cc. of the washed yeast suspension was then added to a series of these suspensions and fermentation allowed to proceed. The temperature of fermentation was again 30°C. Check suspensions containing no yeast were included in each series. During fermentation the H-ion concentration was determined at 15-minute intervals and never allowed to fall lower than the values given. At the proper time the suspension was removed from the water bath, the volume made to 1,000 cc., and the extraction of electrolytes and the determination of the viscosity made in the way described by Johnson, Herrington, and Scott (1929). The results obtained are given in Table IV. By comparing the viscosities of

TABLE IV

RATE OF DECREASE OF VISCOSITY OF FLOUR-WATER SUSPENSIONS ALLOWED TO FERMENT AT DIFFERENT H-ION CONCENTRATIONS. FERMENTATION TEMPERATURE 30°C

Time Digested	Digested at pH 5.65	Digested at pH 6.10	Digested at pH 6.70	Digested at pH 7.25
	Viscosity	Viscosity	Viscosity	Viscosity
	degrees MacM	degrees MacM	degrees MacM	degrees MacM
1 hour no yeast	239	212	144	116
10 hours no yeast	235	214	199	189
1 hour with yeast	186	174	148	118
2 hours with yeast	154	145	179	132
4 hours with yeast	111	124	157	146
6 hours with yeast	94	116	130	156
8 hours with yeast	52	89	114	162
10 hours with yeast	34	52	89	159

the suspensions fermented or digested for 10 hours with or without yeast it is evident that the yeast operated to reduce the viscosity in all cases and that the degree of decrease was greater the more acid were the conditions of fermentation. Thus the viscosity of the suspension allowed to ferment at pH 5.65 was 34, while that of the suspension allowed to ferment at pH 7.25 was 159.

Regular decreases in viscosity occur during fermentation at pH values 5.65 and 6.10, but on allowing the fermentation to proceed at pH values 6.70 and 7.25 the viscosities appeared to increase over that of the suspension digested for 1 hour and containing no yeast. This is doubtless due to the high alkalinity of these suspensions preventing the phytase from liberating soluble electrolytes which would be removed during extraction. As they are not re-

moved they remain behind to depress the viscosity of the finally acidulated suspension. Johnson and Herrington (1928) have already shown the mechanism of operation of this phenomenon. In order to obtain a more correct idea of the changes occuring in the viscosity it would therefore have been necessary at each time interval to determine the viscosity of a check suspension as well as that of a suspension containing yeast. The difference in viscosity between the two values obtained would be a measure of the effect of the yeast fermentation on the flour proteins. This was actually done only for check and yeast-containing suspensions digested or fermented for 10 hours, but from these data it is possible to calculate the probable viscosity at the end of any other definite time of fermentation, or in other words it is possible to calculate the equivalent quantity of protein whose effect on the viscosity has been reduced to 0° MacMichael. In order to bring all the data to more readily comparable terms the viscosity of the suspensions fermented or digested with or without yeast will be multiplied by the ratio

$$\frac{\text{viscosity of suspension digested for 10 hours without yeast at pH 5.65}}{\text{viscosity of suspension digested for 10 hours without yeast at any pH}}$$

This is a legitimate procedure as viscosity-flour concentration curves are logarithmic. That values which are probably correct can be obtained by this method of calculation will appear later. The results of the calculations are given in Table V. The curves in Figure 1 were drawn on the basis of these calculations. The curves show very graphically the changes in viscosity which occurred in extracted and subsequently acidulated flour-water suspensions.

Some evidence has already been given to indicate that the changes in viscosity induced by fermentation with yeast are irreversible, not being dependent on changes in H-ion concentration. In order to substantiate this evidence another experiment was conducted in which flour suspensions prepared in the same manner as the preceding were used. During fermentation no alkali was added to neutralize the acids formed, but one hour before the extraction of electrolytes was begun 75 cc. of buffer solution was added. This brought the pH of the suspension to a point more alkaline than that of the original suspension. Extraction of electrolytes was then conducted and the viscosity determinations made in the usual way. The data in Table VI show that regular decreases in viscosity occurred during fermentation, indicating again that the effect of

TABLE V

CORRECTED VISCOSITIES FROM WHICH CURVES IN FIGURE 1 WERE DRAWN

Conditions of Digestion	Viscosity as Determined	Calculated Viscosity
	degrees MacM	degrees MacM
Digested 10 hours without yeast at pH 5.65	235	235
Fermented 10 hours with yeast at pH 5.65	34	34
Digested 10 hours without yeast at pH 6.10	214	235
Fermented 10 hours with yeast at pH 6.10	52	57
Digested 10 hours without yeast at pH 6.70	199	235
Fermented 10 hours with yeast at pH 6.70	89	105
Digested 10 hours without yeast at pH 7.25	189	235
Fermented 10 hours with yeast at pH 7.25	159	198

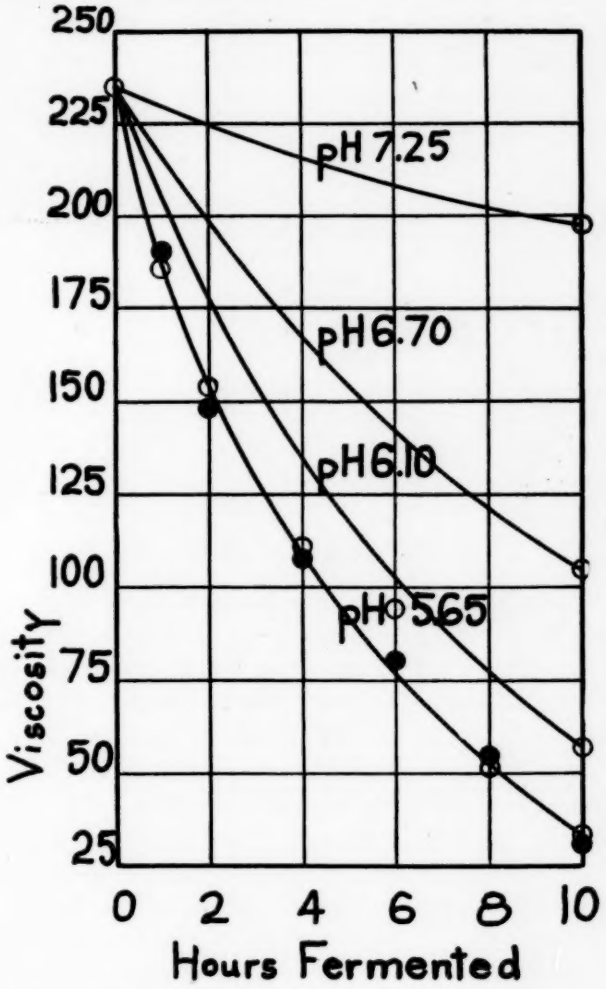

FIGURE 1

Graph Showing Rate of Decrease of Viscosity of Flour Suspensions Allowed to Ferment at Different H-ion Concentrations

yeast fermentation on the flour proteins is irreversible and that the original imbibitional capacity of the flour suspension cannot be restored by neutralization of the acids of fermentation.

Due to the addition of the buffer the viscosities of all the suspensions in this experiment are lower than the corresponding ones in the experiment conducted at pH 5.65 as recorded in Table IV. When the viscosities in Table VI are multiplied by the ratio which has been given, values which agree very well with those in Table IV are obtained. The closeness of the agreement is best shown in Figure 1 (curve pH = 5.65) where the viscosities from Table IV are plotted in open circles and the calculated viscosities from Table VI are plotted in blackened circles. This agreement appears to justify the method of calculation used for certain data in Tables V and VII.

TABLE VI

VISCOSITIES OF YEAST-FERMENTED FLOUR-WATER SUSPENSIONS THE ACIDITIES OF WHICH WERE NEUTRALIZED BY THE ADDITION OF A PHOSPHATE BUFFER BEFORE EXTRACTION AND PREPARATION FOR THE VISCOSITY DETERMINATION

Time Digested	pH		Viscosity	Calculated Viscosity
	before addition of buffer	after addition of buffer		
			degrees MacM	degrees MacM
1 hour without yeast	5.65	6.44	169	229
10 hours without yeast	5.65	6.44	174	235
1 hour with yeast	5.62	6.44	142	191
2 hours with yeast	5.58	6.40	110	148
4 hours with yeast	5.51	6.35	80	108
6 hours with yeast	5.27	6.39	60	81
8 hours with yeast	4.73	6.34	41	55
10 hours with yeast	4.24	6.32	24	32

By use of the method described by Johnson, Herrington, and Scott (1929) it will now be possible to calculate the quantity of protein or its equivalent, the effect of which on viscosity has been reduced to 0° MacMichael by the yeast fermentation. The original viscosities of suspensions containing 12, 16, and 20 gm. of the flour were 32°, 100°, and 235° MacMichael respectively. The viscosities of the fermented suspensions as read from the curves in Figure 1 will be used for the calculations. For example the viscosity of the suspension fermented for 2 hours at pH 7.25 will be taken as 225° MacMichael, etc. A decrease in viscosity of 10° MacMichael has accordingly resulted which at this viscosity level is equivalent to the protein contained in 0.27 gm. of the original flour. The yeast has, therefore, operated to reduce to 0° MacMichael the effect on the viscosity of 0.27 gm. of flour. The data used in making similar

calculations for the rest of the fermentation experiments are given in Table VII. The values given in the fourth column of the table

TABLE VII

DATA USED IN CALCULATION OF QUANTITY OF FLOUR WHOSE EFFECT ON VISCOSITY HAS BEEN DESTROYED DURING FERMENTATION WITH YEAST

Time Fermented	Viscosity as read from Figure 1	Log Viscosity	Log flour conc. as read from orig. visc.-flour conc. curve	Flour conc. as obtained from log in column to the right	Flour whose effect on visc. has been eliminated by fermentation	
hours	degrees MacM			gm.	gm.	%
			Fermented at pH 7.25			
0	235	2.37107	1.301	20.00	.00	.00
2	225	2.35218	1.295	19.73	.27	1.35
4	216	2.33445	1.290	19.50	.50	2.50
6	208	2.31806	1.286	19.32	.68	3.40
8	202	2.30535	1.283	19.19	.81	4.05
10	198	2.29667	1.280	19.06	.94	4.70
			Fermented at pH 6.70			
0	235	2.37107	1.301	20.00	.00	.00
2	198	2.29667	1.280	19.06	.94	4.70
4	168	2.22531	1.261	18.24	1.76	8.80
6	143	2.15534	1.243	17.50	2.50	12.50
8	121	2.08279	1.226	16.83	3.17	15.85
10	105	2.02119	1.210	16.30	3.70	18.50
			Fermented at pH 6.10			
0	235	2.37107	1.301	20.00	.00	.00
2	178	2.25042	1.268	18.58	1.42	7.10
4	135	2.13033	1.237	17.26	2 74	13.70
6	103	2.01284	1.206	16.07	3.93	19.65
8	78	1.89209	1.170	14.80	5.20	26.00
10	57	1.75587	1.140	13.80	6.20	31.00
			Fermented at pH 5.65			
0	235	2.37107	1.301	20.00	.00	.00
2	154	2.18752	1.252	17.87	2.13	10.65
4	111	2.04532	1.215	16.41	3.59	17.95
6	94	1.97313	1.197	15.74	4.26	21.10
8	52	1.71600	1.133	13.59	6.41	32.05
10	34	1.53148	1.086	12.19	7.81	39.05

were obtained from the original log viscosity-log flour concentration curve by reading off the flour concentration corresponding to the log viscosity given in Column 3. The values in Column 4 are merely the antilogs of the logs in Column 3. The values in Column 5 are the differences between these antilogs and 20, the original flour-water concentration. The differences obviously represent the quantities of flour containing the protein whose effect on the viscosity has been reduced to 0° MacMichael during the fermentation with yeast.

The data in Table VII show very clearly the reductions in imbibitional capacity effected by the fermentation with yeast. The

hydrogen ion concentration at which the fermentation was conducted, however, had a marked influence on the rate at which the decrease in viscosity occurred. Thus at pH values 7.25, 6.70, 6.10, and 5.65, the quantities of flour containing the protein whose viscosity was reduced to 0° MacMichael during 10 hours fermentation were 4.70%, 18.50%, 31.00%, and 39.05% of the flour used respectively. These percentages also obviously refer to the percentages of original glutenin which no longer imbibes water on acidulation.

Discussion

The results obtained from this study explain certain of the apparently conflicting data obtained by other investigators. Sharp and Gortner (1924) reported that the viscosities of bread-dough suspensions increased during fermentation, while Johnson and Bailey (1924) observed a decrease in the viscosity of fermenting cracker-dough suspensions. The reason for this apparent discrepancy doubtless lies in the fact that bread doughs contain added NaCl, while in cracker doughs the salt is not added until after about 18 hours fermentation. It would be interesting to know whether salt had ever been added at the beginning of fermentation in cracker doughs and if so whether the reason for the change in time of adding it was in accordance with the results of this paper.

Olsen and Bailey (1925) reported that increasing acidity during fermentation and not proteases contributed by the yeast was responsible for the decrease in water-imbibing capacity of the flour proteins. Our data indicate that while the H-ion concentration to which the flour suspension has been exposed during its preparation for the viscosity determination may have a pronounced effect on the final water-imbibing capacity, washed yeast itself also exerts a marked influence on this property. Thus when the H-ion concentration was kept constant during fermentation, the viscosities of yeast-fermented flour suspensions were always lower than those of flour suspensions maintained at the same H-ion concentration but not containing yeast. The degree to which the viscosity of the yeast-fermented suspension was lower than that of the check suspension depended on the H-ion concentration at which the fermentation was allowed to proceed. The rate of decrease of viscosity was lower the lower the H-ion concentration of the fermenting suspension.

The fact that the rate of decrease of the viscosity is lower at low H-ion concentrations may be of significance in breadmaking

practice. Grewe and Bailey (1927) have called attention to the fact that the best bread can be produced from doughs in which the H-ion concentration has been decreased, provided the diastatic activity and fermentation rate are maintained at an approximately normal level. Their explanation for this result was that the addition of alkali brought the gluten proteins nearer their isoelectric range where improvement in extensibility and gas-retaining capacity resulted. Our work indicates that the addition of alkali to fermenting suspensions effects a decrease in the rate at which the gluten proteins lose their power to imbibe water. This may also be one of the factors operating to produce the better bread obtained by Grewe and Bailey in doughs rendered alkaline with NaOH. The fact that the presence of sodium chloride prevented any decrease in the water-imbibing capacity of the gluten proteins, however, gives more weight to the explanation of Grewe and Bailey than to the one we have offered.

The change which fermentation with yeast has effected in regard to the gluten proteins is a very subtle one. If it were due to proteoclastic activity contributed by the yeast enzymes one would not expect the presence of salt to prevent the action of proteases. It is possible, however, that the presence of salt influences the colloidal condition of the flour proteins so that the yeast proteases cannot act on them. Also if the first stage of proteoclastic activity is concerned with a breaking apart or disaggregation of relatively large protein complexes it may be that the presence of salt prevents this from taking place by stabilizing conditions for the existence of large aggregates. As salts are known to be protein precipitants such an explanation is not improbable.

Conclusions

Fermentation of flour suspensions with bakers' yeast effects a marked decrease in the viscosity of the suspension.

Rate of decrease of the viscosity depends on the H-ion concentration during fermentation, high concentrations of the H-ion increasing and low concentrations decreasing the rate of decrease in viscosity.

The presence of NaCl and phosphate buffer salts prevents the decrease in viscosity during fermentation periods as long as 10 hours.

The decrease in viscosity which occurs in flour suspensions not containing added salts is irreversible, the original viscosity not be-

ing restored on the addition of either salts or alkali sufficient to neutralize the acids of fermentation or both.

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YEAST TESTING [1]

W. H. Cook and J. G. Malloch
University of Alberta, Edmonton, Canada

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Introduction

In view of the efforts of the Committee on Standardization of the Experimental Baking Test, of the American Association of Cereal Chemists, to devise a suitable test for the uniformity of yeast, our experience with this type of work will be of interest. At the time this work was undertaken large loaves (350 gm. flour) were used for test baking in this laboratory. It was desired to ascertain the variability of the yeast as a possible factor in the variation between replicate loaves baked from the same flour. Also since two brands of yeast were available it was necessary to make a selection on the basis of uniformity.

Apparatus

After careful consideration of the alternative procedures for making a test of yeast activity it was decided that the measurement of the carbon dioxide produced from sugar by a definite quantity of yeast acting in a liquid medium was the most feasible. Slator (1906) and other workers have reported the variation in yeast activity with temperature, and Harden (1914) and Slator (1906) have emphasized the necessity of continuous shaking of the flask containing the test mixture in order to release the gas from the solution and to keep it in equilibrium with the space above. Accordingly an apparatus was devised which permitted the continuous shaking of the reaction flasks in a constant temperature bath set at 28° C. (the fermentation temperature then used in test baking). The apparatus is shown diagrammatically in Fig. 1.

Reaction Medium

Maltose was selected as the sugar substrate since it is the sugar produced in dough by the action of diastase on starch. Slator (1906) reports that the rate of fermentation is practically independent of the sugar concentration except in very dilute solutions, and a review of his data led to the use of an 8% solution in order to ensure an excess. Preliminary tests of the effect of the presence of a number of salts in the medium showed that phosphates were the

1 Paper No. 10 of the Associate Committee on Grain Research, National Research Council of Canada.

only salts that greatly accelerated the velocity of fermentation measured over a two-hour period. This fact might be used as an argument against the use of phosphates in the substrate but it seemed desirable to measure the maximum activity of the yeast. A further reason for the use of phosphates lies in their buffer action.

The pH of a normal fermenting dough is usually in the region of 5.0 to 5.5 and in a test such as this it seemed desirable to duplicate this condition as closely as possible. Chabot (1923) found that the rate of fermentation increased with acidification up to pH 4.3,

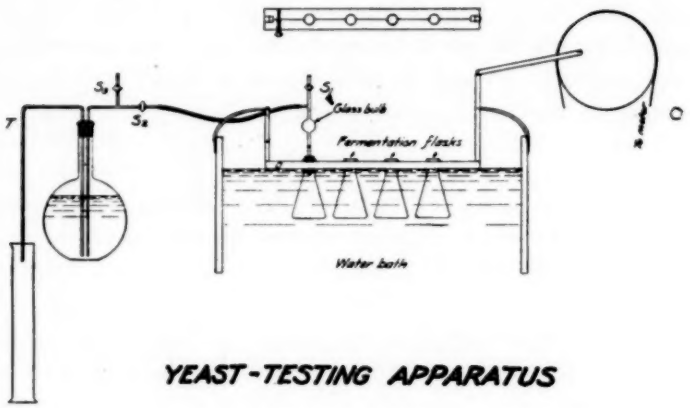

Figure 1. Diagram of Apparatus.

the most acid condition tested. Willstätter and Bamann (1926) found that the optimum pH for the fermentation of maltose was 4.5, but that over the wide range of pH 3.0 to pH 6.5 there is only a small variation in rate. Organic buffers seemed unsuitable for controlling the acidity, as some are themselves fermented while some are toxic to the yeast. The use of phosphates in the nutrient medium seemed therefore to be justified. Various phosphatic salts were then tried in different concentrations and mixtures.

The choice finally rested on a mixture containing 2.5 gm. Na_2HPO_4 to 10 gm. KH_2PO_4 in 1 liter of solution. It was found that when the maltose was mixed with the buffer solution and allowed to stand there was considerable fermentation by foreign organisms even at room temperature. Accordingly the maltose and the buffer solutions were made up separately in double concentra-

tion and equal portions of each solution were mixed just before making each test.

Effect of Fermentation on Acidity

An experiment was carried out to determine the change in acidity using this medium. Details of the experiment will not be given here as the procedure followed was essentially that described in the adopted method. The acidity was determined potentiometrically using a hydrogen electrode. The results are given in Table I.

TABLE I

EFFECT OF FERMENTATION ON ACIDITY

Test No.	Acidity of medium and yeast. Initial	Final	Fermentation Period
	pH	pH	
1	5.6	4.6	2 hours
2	5.6	4.6	"
3	5.6	4.8	"
4	5.6	4.7	"
5	5.6	5.2	1 hour
6	5.5	5.2	"
7	5.6	5.1	"
8	5.6	5.2	"

It will be seen that the initial acidity is quite constant at pH 5.6. In one hour the pH fell to 5.2 and in two hours to 4.7. A blank determination showed practically no change. As the change during one hour falls within the limits of the acidity of fermenting dough this period appeared to be quite satisfactory.

Effect of Time on Gas Production

In order to arrive at a final decision on the length of test, periodic measurements of the gas production were made using the same procedure as before. Fifteen samples were used and in all cases the quantity of gas produced in one hour was almost exactly half that produced in two hours. The results for the two samples reported in Table II are typical of the whole series.

As shown by the above data the first 30 min. interval was the lowest in gas production followed by an accelerated activity in the second period. The two remaining intervals were intermediate in value between these two extremes. These results are in harmony with those quoted by Harden (1914), who found accelerated fer-

TABLE II

EFFECT OF TIME ON GAS PRODUCTION

Fermentation interval	Gas produced	Gas produced per 30 min.	Ratio Gas produced 1 hour. / Gas produced 2 hours.
min.	cc.	cc.	
	Sample I.		
30	153	153	
60	360	207	
90	534	174	
120	720	186	0.50
	Sample II.		
30	184	184	
60	426	242	
90	649	223	
120	873	224	0.49

mentation during the initial period in the presence of phosphates. When these results are considered as two one-hour intervals however, it can be seen that they are practically identical in gas production as shown by the ratio of the one to the two-hour period. The values for the first 30 min. are decidedly below that for any other similar period. The one-hour period was accordingly adopted.

Adopted Method

Sampling. A representative sample of yeast is taken from the block and suspended in sufficient water to give 4 gm. of pressed yeast in 10 cc. water. The amount of suspension made up is in all cases greater than that required for a duplicate determination.

Medium. Sugar solution—16% Maltose dissolved and filtered.
Buffer solution—5 gm. Na_2HPO_4 and 20 gm. KH_2PO_4 in 1 liter of solution.

Using the apparatus previously described the following procedure is carried out:

Two fermentation flasks are placed in the clamp in the thermostat (at 28° C.) and 50 cc. of the maltose solution added to each. A 10 cc. graduate, previously cut off at the 10 cc. mark, is immediately filled to overflowing with a portion of the well-shaken yeast suspension. This technique avoids possible errors due to the suspension settling. The yeast is then transferred to the fermentation flasks and the graduate thoroughly rinsed with 50 cc. of the buffer solution. The flasks are then connected to the apparatus and the shaker started. The shaking is carried out at such a rate that a further increase in speed does not increase the velocity of gas production. Five minutes is allowed for the solution to attain the temperature of the thermostat, and during this period the stop-

cock s_1 is left open to maintain atmospheric pressure in the system. Meanwhile the liquid conveying tube t is filled with liquid by blowing gently throughout stopcocks s_3 with stopcock s_2 closed, s_3 then being closed. At the end of the five-minute period s_1 is closed and s_2 opened to allow the gas to pass over. The test is allowed to run for 1 hour.

Gas Production of Different Yeast Samples

Using the above method the first experiment undertaken was to test the yeast which was being used daily in the baking experiments. Two commercial yeasts produced by different companies were available and a number of samples from both of these were tested. For the purposes of this paper they have been designated as 'A' and 'B' yeasts. At the time of conducting this experiment, the 'A' yeast was being used in the baking tests so this product was the first subject of study.

It was found on testing, that the gas production of different samples of this yeast varied a good deal and the study was therefore extended to a number of samples of the 'B' yeast. The results obtained from duplicate one-hour gas-production tests of a number of these samples is given in Table III. As shown by this data the 'B' yeast is much less active than the 'A,' but has the advantage of being much more constant.

TABLE III

GAS PRODUCTION OF DIFFERENT YEAST SAMPLES

'A' Yeast		'B' Yeast	
Sample	Gas Produced in 1 Hour	Sample	Gas Produced in 1 Hour
	cc.		cc.
AT1	416	BT1	201
AT2	340	BT2	193
AT3	344	BT3	224
AT4	289	BT4	222
AT5	362	BT5	221
AT6	296		
AT7	363		
AT8	320		

Effect of Storage on Gas Production

Both these yeasts were known to have been stored a certain length of time before being received at the laboratory. Further, since the 'B' yeast in all cases must have been in storage longer, it was considered that this might account for its lower gas producing power. An experiment was therefore conducted to test the effect of storage at about 0° C. in an electrical refrigerator. The average results of duplicate tests are given in Table IV.

As shown by these figures there was little change in most of the samples for periods up to ten days, when they were kept in the original blocks. When stored in suspension, however, the activity seemed to fall off more rapidly but the data are meagre on this point. As these results show there is little chance of the yeast samples losing their normal activity even over comparatively long intervals, the differences previously found must be the result of other factors.

TABLE IV

EFFECT OF STORAGE ON GAS PRODUCTION

Sample	Initial Gas Production	Length of Storage Period	Final Gas Production
	cc.	days	cc.
	Samples stored in the pressed blocks.		
AS1	420	4	412
		5	426
		10	410
AS2	362	6	304
AS3	357	1	362
BS1	202	1	198
	Samples stored in suspension.		
ASS1	342	1	356
ASS2	289	1	172

Effect of Yeast Concentration on Gas Production

An experiment on the effect of concentration was performed using two samples of yeast A and one sample of yeast B. The results are given in Figure 2.

It will be noted that the 'A' and 'B' yeasts gave lines of different slopes and that samples of yeast A with different initial activities gave parallel curves.

Relation of Gas Production to Rate of Rising of Dough

Having established a linear relationship between the concentration of the yeast and the CO_2 produced in a test by the adopted method it seemed desirable to investigate the relationship existing between this value and the rate of rise of dough. Saunders (1921) points out that "two different samples of yeast evolving the same amount of carbon dioxide in the same length of time, may behave quite differently when used in the making of bread." In order to avoid complications due to variation in gas-retaining capacity, one flour (a commercial patent) was selected as a standard. The absorption of this flour was determined and a number of doughs made from it allowed to ferment for different lengths of time. This experiment led to the conclusion that short fermentation periods in the region of 70 min. were more reliable for measure-

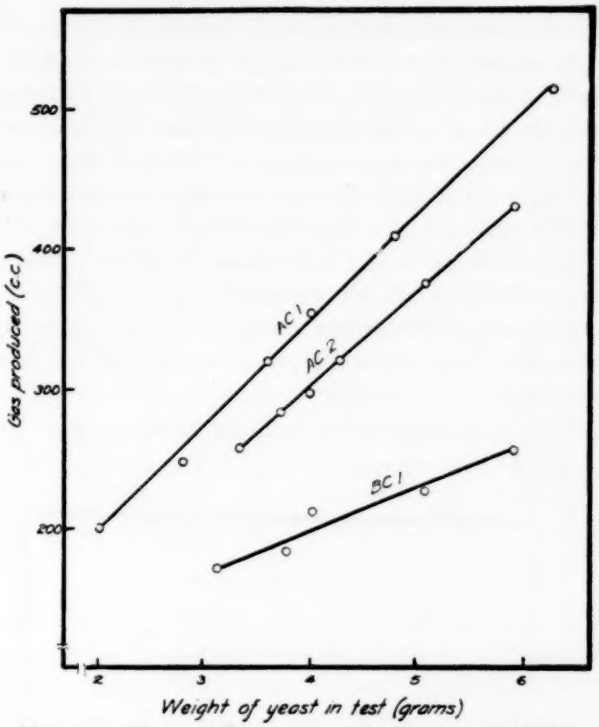

Figure 2. Effect of Yeast Concentration on Gas Production

ments of the rate of rising with this flour than the usual fermentation period of 90 min. or more. With fermentation periods above 70 min. it was found difficult to obtain concordant results from duplicate samples. This was probably due to a decrease in the gas-retaining capacity. A 65 min. period was chosen. Using this standard flour a suitable rate of fermentation was determined and set as an arbitrary standard for checking other yeast samples. The method used was to mix a dough using 350 gms. flour in the regular way used for test baking and to vary the yeast concentrations. From this dough two 250 gm. portions were scaled, and placed in slightly greased one-liter tall-form beakers graduated at a volume of 750 cc. These were allowed to ferment at 28° C. and the time required for the dough to reach this volume noted. The adopted rate of fermentation for this flour was that which would raise a 250 gm. dough to a volume of 750 cc. in 65 minutes at 28° C., this being the desired rate as determined by subjecting a similar loaf to the complete baking process.

The result of plotting the gas production against the time required to raise the dough, for the same yeast concentrations as

were used in the previous experiment, is shown in Fig. 3. In this study another patent flour of lower protein content was included, the rising period, determined as before, for this flour being 95 minutes. As will be seen in the curve a 65-min. fermentation period with the first flour corresponds closely with a 330 cc. gas production for the 'A' yeast, while this gas production cuts the curve obtained for the second flour in the region of 95 minutes. The 'A' and 'B' samples again show a big difference in slope, both of course being negative in direction. A unit increase in gas production in the 'B' yeast reducing the time required to raise the dough two to three times that of the 'A' yeast, showing the 'B' to be more efficient in the dough.

Having this data available, a number of 'A' yeasts were tested for gas production and corrected to give the desired rate of rising

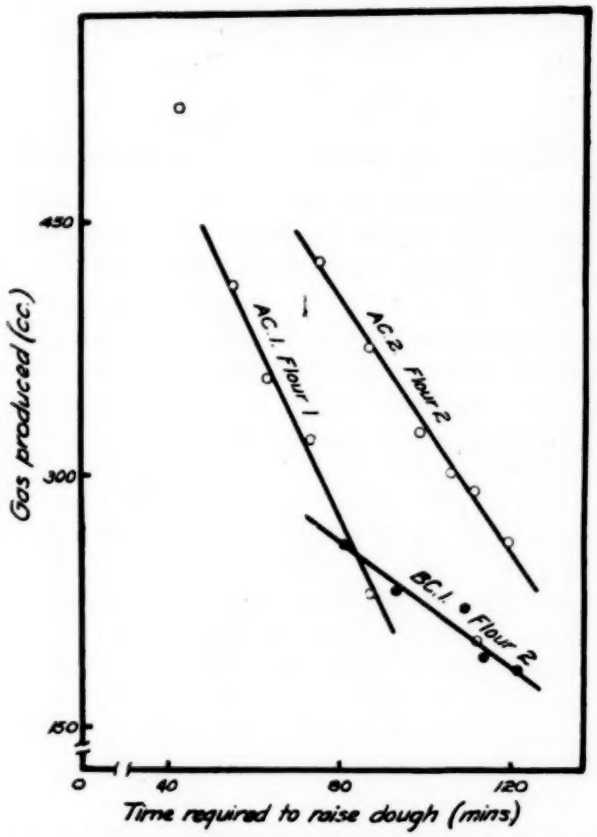

Figure 3. Relation of Gas Production in Liquid Medium to Time Required to Raise a Standard Dough

in the dough, using Flour 1. Over a number of samples it was found possible to correct it to 65± 4 minutes with most of the tests quite close to 65 minutes. The adjusting of the yeast samples used in the test baking was then undertaken. The technique followed was to make up the yeast suspension in twice the concentration formerly used in the bread, and to use 5 cc. of this for the gas test. From the result obtained the amount of dilution necessary to give the proper amount of yeast in 25 cc. of suspension was calculated and this amount used in the loaf. A further check was obtained by running a dough test as the first loaf of each series, this giving an indication as to the efficiency of the correction. When experimental baking was undertaken with concentration of the 'A' yeast corrected it was evident that a lower variability occurred from day to day.

However, since the 'B' yeast showed very much less variation in gas production than the 'A' yeast it was used in all subsequent test baking.

Discussion

The introduction of the 100 gm. loaf for test baking made more replicates possible and in view of the small number of times that it was necessary to correct for variations in the 'A' yeast it was considered easier to rebake the loaves to obtain check volumes than it was to continue the daily yeast test. However, the procedure outlined in this paper proved to be very useful when yeast of uniform activity was not available.

It should be pointed out that the uniformity of the yeast from day to day may be checked very simply by use of the dough check only. This eliminates the labor and time involved in making the gas production test and the standard conditions of the test, i.e. time of rise and final volume may be readily adapted to the requirements of any particular laboratory. It is hardly suitable, however, for checking the yeast used in a number of laboratories.

As a result of the investigations outlined in this paper the authors are of the opinion that the following points should be considered by the Committee in formulating a method which will be general in its application.

1. The test should be made at a definite and controlled temperature.

2. If a liquid medium is used, continuous shaking of the reaction flask is absolutely essential.

3. The acidity of the medium may vary within fairly wide limits without affecting the gas production appreciably, but it

would seem to be advisable to use a buffer in the medium so that the limits of variation are known.

4. Under the conditions which we used in our work, measurements of gas production over periods of less than one hour were unreliable.

5. The relative efficiency in dough of two strains of yeast is not necessarily the same as their relative gas production in liquid media. There is a possibility that the stimulating effect of phosphate in the liquid medium may be responsible for the discrepancies between gas production in liquid media and the efficiency in dough noted in our experiments. If a gas production test is to be useful for purposes of standardization the variation in gas production with yeast concentration must be of the same order as the variation in rate of rising with concentration.

6. In order to eliminate the apparent variation in the characteristics of different strains of yeast the committee might consider the advisability of arranging with a firm of yeast manufacturers to place a special yeast for experimental baking on the market.

7. The moisture content of the yeast may be an important variable.

Summary

1. The experience of this laboratory in testing yeast by gas production and by rate of rising of a standard dough is outlined.

2. Suggestions of points to be considered in formulating a method for testing yeast are made.

Acknowledgment

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THE ADAPTABILITY OF THE QUINHYDRONE ELECTRODE TO CEREAL WORK

L. V. SORG

Larabee Flour Mills Co., Kansas City, Mo.

(Read at the Convention May, 1929)

The hydrogen electrode, as a means of determining the H-ion concentration of flour extracts, presents a series of problems in manipulation, which, even with carefully incorporated refinements, makes the system cumbersome. One of the greatest of these to the busy chemist, is the length of time required by the system to reach equilibrium. During the time required to reach equilibrium, many things can happen to the solution itself to change the H-ion concentration. Thus, a system which would reach equilibrium very quickly would eliminate the chance for error presented here. Again, the hydrogen electrode is subject to considerable variation from temperature and pressure, each of these factors having a measureable effect on the H-ion concentration. The system in which these two factors have the minimum effect will be the most desirable. From the standpoint of cleanliness, the bubbling type of hydrogen electrode makes a bad case with the soluble protein in flour extracts. Then too, the platinum electrode used requires frequent plating and unless the electromotive force and the current density in subsequent platings are the same, the character of the deposited metal will vary and it is the belief of many that this is the foundation of certain errors. This paper presents the effort to overcome some of these factors entirely and to reduce others to a minimum, as well as to determine the accuracy of such a system and factors affecting it.

The nature of this work consisted first, in the determination of the ability of the system:

Au, Quinhydrone, solution x || KCl (saturated), HgCl, Hg

to produce the same results as the system:

Pt, H_2, solution x || KCl (saturated), HgCl, H_2, Pt

Then followed a study of the way in which the above mentioned factors affected this system.

Haber and Russ (1904) were the first to set up a system in which quinhydrone was one of the components. In 1904 they measured the potential of an electrode formed from a solution of quinhydrone in a mixture of sulfuric acid, water, and ethyl alcohol.

However, this system is complex and they were never able to reproduce the potential. In 1920 Biilman set up a system of:

Pt, Quinone, Hydroquinone, 0.1N HCl || 0.1N HCl, H_2, Pt

which he studied extensively. From his work he was able to show that quinhydrone (a compound containing an equimolecular quantity of quinone and hydroquinone) could be used in place of the hydrogen electrode for H-ion determinations.

The theory of the quinhydrone electrode is based upon the fact that quinhydrone yields the active agents of the electrode after the following equation:

$$\text{Quinhydrone} = C_6H_4O_2 + C_6H_4(OH)_2$$

It is significant that the quinone and hydro-quinone are present in equimolecular quantities, i.e, their molecular ratio is 1. Likewise the active reagents dissociate after the following equation of equilibrium:

$$C_6H_4O_2 + H_2 \rightleftarrows C_6H_4(OH)_2$$

It will be seen that a definite quantity of hydrogen is represented and if we consider this reaction occurring at a definite molecular concentration, the following results:

$$[H_2] = K \frac{[C_6H_4(OH)_2]}{[C_6H_4O_2]}$$

The brackets represent molecular concentrations and K is the equilibrium constant at a given temperature. From this it is seen that the concentration of hydrogen at a given temperature is dependent upon the ratio of the molecular concentrations of quinone and hydroquinone and not upon their absolute concentration. The hydrogen resulting from the dissociation represents, at a given temperature, a constant but infinitesimal pressure of hydrogen (P_q). Then

$$P_q = K \frac{[C_6H_4(OH)_2]}{[C_6H_4O_2]}$$

but

$$\frac{[C_6H_4(OH)_2]}{[C_6H_4O_2]} = 1$$

hence

$$P_q = K$$

Thus the quinhydrone electrode is in reality a hydrogen electrode where the pressure of hydrogen instead of being one atmosphere is P_q. It is because of this pressure of hydrogen, extremely low but at the same time very constant, one is able to use the quinhy-

drone electrode in the determination of H-ions. In considering the gas chain

Au, Quinhydrone, 0.1N HCl || 0.1 HCl, H_2, Pt

the single electrode potential (E_q) of the quinhydrone electrode is

$$E_q = \frac{RT}{nF} \log_e \frac{P_q}{p}$$

where P_q is the pressure of hydrogen from the dissociation of the quinhydrone and p is the osmotic pressure of the ions in the electrolyte (0.1N HCl). Likewise for the hydrogen electrode we have,

$$E = \frac{RT}{nF} \log_e \frac{P_H}{p}$$

the potential of the entire chain is:

$$E - E_q = \frac{RT}{nF} \log_e \frac{P_H}{p} - \log_e \frac{P_q}{p}$$

but the electrolyte is the same in both electrodes hence $p = p$ and we have:

$$E - E_q = \frac{RT}{nF} (\log_e P_H - \log_e P_q)$$

At 25° C., $E - E_q = 0.699$ volts and $P_q = 10_{-23.34}$ atmospheres.

For evaluating the H-ion concentration of a solution from the observed e.m.f. with the quinhydrone electrode the same formula used for the hydrogen electrode is applicable.

It will be remembered that the osmotic pressure p of a solution is proportional to the molecular concentration C (in the case of ionic concentrations C^+); also that the inert electrodes exhibit no solution pressure

$$E - E_o = \frac{RT}{nF} \cdot \log_e 1/C_{H^+}$$

or

$$E - E_o = 0.000198T \log 1/C_{H^+}$$

$$\log 1/C_{H^+} = pH = \frac{E - E_o}{0.0001984T}$$

E = observed voltage

E_o = normal potential of system

The apparatus used in this work consisted of a Type K Leeds and Northrup potentiometer balancing a Weston standard cell. The usual saturated calomel half-cell was used. Several inert electrodes were tried, among them were the following: both platinum plate and wire, gold-plated platinum, gold wire, and the Bailey electrode.

It was impossible to make a study of the effect of extreme pressures on the H-ion concentration when measured with the quinhydrone electrode. But over the long period of time during which the measurements were taken, accurate data were kept of barometric pressure.

The study of the effect of temperature was carried out in an incubator, the temperature of which could be controlled to ± 0.5 degree. The solution under examination as well as the calomel half-cell were placed within the incubator and sufficient time allowed for the equilibrium to be established at that temperature. As a general rule, the solutions used were placed in the incubator so that they could remain there during the night. When the measurements were to be made, the quinhydrone was added and the measurements taken. This was done in order to prevent any secondary reactions which might have occurred had the quinhydrone been added to the solution and then allowed to stand in contact with it.

The type of calomel half-cell vessel used was developed by the author and seemed especially suited for this work. It resembles the new Leeds and Northrup electrode vessel but contains in addition to the diffusion cup, an ungreased stop-cock to prevent the least possibility of any diffusion into the calomel cell which would affect its accuracy. In order that a fresh liquid junction might be had, the cell was flushed out following each determination.

It was essential that all apparatus be kept extremely clean and as free as possible from exposure to laboratory fumes.

The general procedure consisted as follows: 20 cc. of solution to be tested was placed in a small beaker of about 150 cc. capacity. To this was added a very small amount of powdered quinhydrone (0.1 gm.). The solution was stirred for about one minute, and was then placed in contact with the gold electrode and the calomel half-cell by immersing both of them in the same. The potentiometer was then adjusted to the null point as indicated by a galvanometer and the readings recorded.

It would be futile to study any system which primarily did not produce results comparable to those with the hydrogen electrode. Hence, the first step is to determine the agreement between the two systems, that of the hydrogen electrode and that of the quinhydrone electrode. The following are the gas chains which were checked

Hg, HgCl, KCl || Pt (Black), H_2
Hg, HgCl, KCl || Au, Quinhydrone

Table I shows the agreement between the two systems. This table represents a composite of a large number of determinations. It will be seen that the greatest difference on any single determination is 0.03 units pH and the average difference is 0.00769 units pH. The average deviation from the mean is 0.00384. It is quite evident then, that the results obtained in using the quinhydrone electrode in place of the hydrogen electrode are reliable within the limits of experimental error and to a greater degree than one is able to interpret.

TABLE I

AGREEMENT BETWEEN THE HYDROGEN ELECTRODE AND THE QUINHYDRONE ELECTRODE

Hydrogen pH	Quinhydrone pH	Difference pH	Deviation from mean
6.09	6.07	—0.02	—0.012
6.09	6.07	—0.02	—0.012
5.92	5.92	0.00	0.000
5.92	5.90	—0.02	—0.012
5.95	5.95	0.00	0.000
5.42	5.42	0.00	0.000
4.85	4.84	—0.01	—0.002
5.58	5.58	0.00	0.000
5.98	5.95	—0.03	—0.022
3.80	3.80	0.00	0.000
0.746	.746	0.00	0.000
2.48	2.48	0.00	0.000
3.70	3.70	0.00	0.000
		Av-0.00769	Av-0.00384

The time factor involved in making a determination of the hydrogen-ion concentration is important in routine work. Figures I and II show curves of saturation time. The time is plotted as the abcissae and the electromotive force in millivolts as ordinates. In the majority of cases a constant potential is not reached until after ten minutes when the hydrogen electrode is used. Opposed to that is approximately one minute which is required for the quinhydrone electrode to reach a constant potential. In extreme cases the time has been as low as ten seconds. It should be stated though that the quinhydrone was in contact with the solution exactly one minute while being stirred before the measurement was taken. It is safe to say that a constant potential will be attained by the quinhydrone electrode as fast as the operator can manipulate the apparatus.

Halton and Fisher (1928) have noticed a drift of potential in using the quinhydrone electrode. It will be seen from the curves that no such drift occurred. It has been possible at many times to

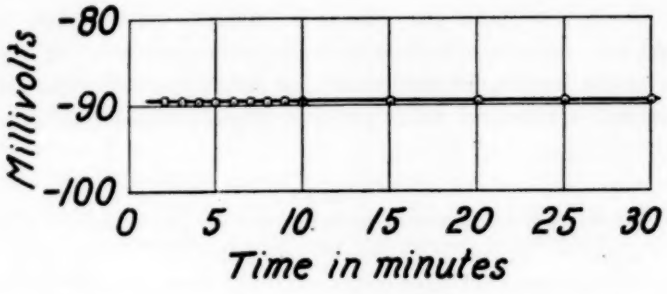

Fig. 1. Saturation time and drift of potential. Quinhydrone Electrode.

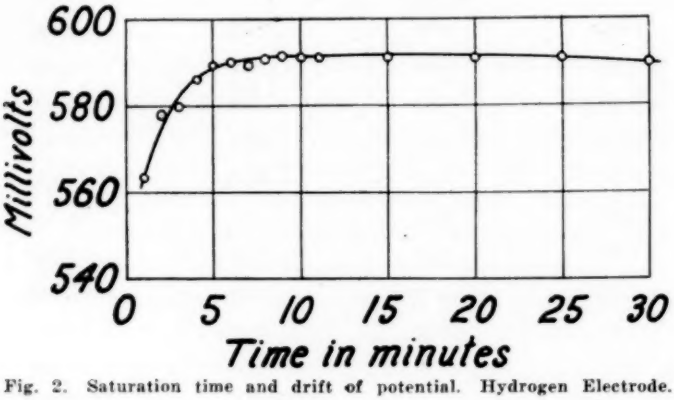

Fig. 2. Saturation time and drift of potential. Hydrogen Electrode.

maintain a constant potential within three millivolts for three hours. This was not possible with the hydrogen electrode but the author is at a loss to explain such a drift when using the hydrogen electrode. Veibel (1923) conducted measurements lasting up to one hundred and forty-four hours and found that the variation was only 0.8 millivolts, which is well within the limits of accuracy obtainable in flour-water extracts.

The quinhydrone electrode is subject to temperature variation the same as is the hydrogen electrode. Table II, which is due to Biilman and Krarup (1924), shows a variation of the system:

Pt, Quinhydrone, 0.1N HCl || 0.1N,HCl,H_2,Pt.

Many times the author has duplicated this determination at 25° C. From these it is easy to calculate what effect any change in tem-

perature will have upon the observed electromotive force, or the following formulae may be used:

$$\pi_t = 0.7175 - 0.00074t$$

Where π_t = e.m.f. at t°C

—0.00074 is the temperature coefficient of the quinhydrone electrode.

TABLE II

EFFECT OF TEMPERATURE ON THE QUINHYDRONE ELECTRODE.

Temp. °C.	Calc. e.m.f., volts	Obs. e.m.f., volts
0	0.7175	0.7173
18	0.7042	0.7044
25	0.6990	0.6990
37	0.6901	0.6901

For routine determinations, the variation of temperature has no interpretable effect upon the H-ion concentrations of flour-water extracts. Within the limits of accuracy, the potential temperature curve of the quinhydrone electrode is a straight line.

The hydrogen electrode is subject to error unless the pressure of the hydrogen is corrected both for atmospheric pressure and

TABLE III

EFFECT OF PRESSURE ON pH USING QUINHYDRONE ELECTRODE

Pressure M. M.	e.m.f. volts	pH
730.4	—.221	3.90
731.8	—.221	3.90
735.0	—.219	3.94
726.5	—.221	3.90
732.0	—.218	3.97
731.1	—.219	3.94
721.9	—.221	3.90
734.0	—.221	3.90
729.9	—.219	3.94
723.0	—.220	3.93
737.7	—.220	3.93
732.2	—.220	3.93
719.4	—.220	3.93
729.0	—.220	3.93
733.3	—.221	3.90
727.5	—.220	3.93

vapor pressure. The quinhydrone electrode does not show an appreciable error from pressure effect as is shown in Table III. Thus ordinary pressures have no effect upon this system.

It has been stated that good agreement could not be attained on duplicate determinations when the quinhydrone electrode was used. Table IV is an effort to determine what can be expected on duplicate determinations. The largest difference obtained was 3.1 millivolts, which is greater than four times the average deviation from the mean and is therefore discredited. Even when this is included, the average difference from duplicate determinations is 0.802 millivolts, which is an excellent agreement attained by Veibel. To show the reliability of these results, the average deviation from the mean is 0.358 millivolts, which is approximately half of the mean. These errors between duplicate determinations are beyond interpretation. They also speak for the reliability of the quinhydrone electrode.

TABLE IV
AGREEMENT BETWEEN DUPLICATE DETERMINATIONS

1 e.m.f. volts	2 e.m.f. volts	Difference, volts	Dev. from mean
—0.12992	—0.12992	0.00000	0.00000
—0.13580	—0.13372	0.00204	+0.00124
—0.12537	—0.12421	0.00116	+0.00036
—0.14247	—0.14313	0.00066	—0.00014
—0.14021	—0.14173	0.00155	+0.00072
—0.12530	—0.12200	0.00310	+0.00230
—0.11270	—0.11280	0.00010	+0.00020
—0.10057	—0.10057	0.00000	0.00000
—0.10588	—0.10588	0.00000	0.00000
—0.10588	—0.10588	0.00000	0.00000
—0.14281	—0.14100	0.00181	+0.00101
—0.10057	—0.10057	0.00000	0.00000
—0.10681	—0.10650	0.00031	—0.00049
—0.10767	—0.10767	0.00000	0.00000
—0.10105	—0.10000	0.00105	+0.00025
—0.10682	—0.10790	0.00108	+0.00028
		Av. 0.000802	Av. +.000358

It is well worth knowing what type of inert electrodes are most suitable for use in quinhydrone solutions. In true solutions,

the bare platinum electrode is quite suitable, but when working with colloidal solution and more particularly with solutions which contain suspended materials, the bare platinum is unsuitable as will be seen by Table V. Halton and Fisher (1928) and Watson (1927) found this same condition to be true, when bare platinum was used. The very first time a new platinum electrode is used it will give the true H-ion concentration, but during successive determinations, it gradually becomes inefficient and finally useless. Nothing has been found yet which will restore its usability. Nos. 5, 6, 7, and 8 of Table V, show this drift of a new platinum electrode on successive determinations. In this research, the gold electrode was found to be the most suitable. Gold-plated platinum was also equivalent to pure gold. The most satisfactory form of the gold wire electrode is that manufactured by the Leeds and Northrup Co.

TABLE V

COMPARISON OF INERT ELECTRODES

No.	Au pH	Pt pH	Au-Pt pH	Bailey pH
1	5.95	5.53		
2	5.87	5.64	6.20	(See note below.)
3	5.98	4.95		
4	5.87	5.73		
5	5.87	5.87		
6	5.87	5.80		
7	5.87	5.64		
8	5.87	5.73		
9	5.75		5.73	
10	6.02		6.00	
11	5.87		5.87	
12	3.96		3.95	
13	4.02		4.00	

In using the Bailey electrode the diffusion cup had to be removed from the calomel electrode cell. Diffusion resulted so no adequate data could be taken.

Kolthoff found that certain proteins have a marked effect upon the quinhydrone electrode, in some cases producing gross errors. To determine if such could be the case with flour-water extracts, the H-ion concentration was determined on boiled and unboiled extracts. This data is seen in Table VI. The differences obtained are not within the limits of interpretation, which indicate that there is no protein error with the quinhydrone electrode.

TABLE VI

EFFECT OF WATER-SOLUBLE PROTEIN

Per cent extr.	Protein %	pH on extr.	pH on boiled extr.	Dev.
10	2.07	6.02	6.00	—0.02
10	1.83	5.73	5.68	—0.05

In the light of these facts, the quinhydrone electrode may be substituted for the hydrogen electrode in the determination of H-ion concentration in flour-water extracts.

Summary

The quinhydrone electrode offers excellent agreement with the hydrogen electrode. Such variations as do exist are beyond the limits of interpretation.

The quinhydrone electrode surpasses by far the hydrogen electrode in saving time. The equilibrium is reached as rapidly as the operator can manipulate the apparatus.

The quinhydrone electrode suffers a temperature variation as does the hydrogen electrode. The coefficient of temperature is —0.00074 volts per degree Centigrade.

At ordinary laboratory conditions, variations of pressure have no measureable effect upon the quinhydrone electrode.

Duplicate determinations agree within 0.000802 volts, which is beyond interpretations in units of pH.

For flour-water extracts, bare gold wire has been found as the most suitable inert electrode. Bare platinum is satisfactory for most solutions free from suspended matter.

The water-soluble protein in flour-water extracts has no effect upon the H-ion concentration when determined by the quinhydrone electrode.

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CERTAIN EFFECTS OF VARYING THE HYDROGEN ION CONCENTRATION OF THE TEMPERING WATER UPON THE WHEAT PROTEINS[1]

MAX C. MARKLEY
Slater Mill and Elevator Co., Slater, Mo.

Introduction

The tempering of wheat is one of the most important steps in the milling process. Its development has been mainly by rule-of-thumb methods, rather than by scientific research. A search of the available literature failed to reveal any scientific work bearing directly upon the subject. So a research, of which this paper is a portion, was undertaken to discover what chemical changes occur during tempering, and to ascertain their laws of behavior. The object of this particular phase of the work was to inquire into the effects of varying the H-ion concentration of the tempering water upon the simpler nitrogen compounds of the wheat.

Experimental

Four samples of wheat were selected for this work. One was a sample of Hybrid 128 wheat. This is a soft white club wheat having a very low protein content. This sample originated at Fulton, in the Willamette Valley of western Oregon. The second sample was Soft Federation wheat. This wheat was grown at Helix in Umatilla County, Oregon, which is in the northeast corner of the state. A blend of central Missouri soft red winter wheat was used for the third sample. The fourth sample was from a car of low protein hard red winter wheat grown in the Arkansas Valley near Garfield, in Pawnee County, Kansas.

Six 200 gm. portions of each of these wheats were placed in pint Mason jars and 10% respectively of distilled water, 0.1N H_2SO_4, 0.1N NaOH, 0.01N H_2SO_4, 0.01N NaOH, and 0.05N Na_2SO_4 were added. The jars were covered without rubbers, and the contents were thoroughly shaken for several minutes. The jars were then set in an air thermostat at 30° C.

At the end of 20 hrs., and again at 92 hrs., samples were withdrawn and analyzed for moisture, water-soluble nitrogen, titrable acidity, amino-acids by Sørensen's formol titration, and non-protein nitrogen by Ritthausen's copper precipitation method. Each of the dry wheat samples was analyzed in a similar manner.

[1] An extract from a thesis presented to the University of Kansas in partial fulfillment of the requirements for the degree of Chemical Engineer.

Titrable Acidity

The first point of interest was to determine the effect of the different tempering solutions upon the titrable acidity of the wheats, as compared to titrable acidity of the samples tempered with distilled water. The 20 hr. results were very consistent. In all four samples the 0.1N H_2SO_4 increased the titrable acidity to about the calculated amount. The 0.1N NaOH, however, decreased the acidity of the soft red winter wheat only about 75% of the calculated amount, and the hard red winter wheat about 40%. On the Oregon white wheat samples the 0.1N NaOH gave the same results as the distilled water, indicating that all of the alkali had gone into chemical combination with the wheat.

The 0.01N and the salt solutions were rather erratic. At 92 hrs. the results were much the same as at 20 hrs., with no consistent changes. The NaOH probably combined with the proteins, but some may have saponified a portion of the fatty bodies in the embryo. The samples tempered with the 0.1N NaOH were much slower filtering than the other samples.

Amino Acids

The Hybrid 128 wheat showed an increase in amino acid content during 20 hrs. tempering with distilled water. The sample was too small to permit tempering with all solutions to 92 hrs. The 0.01N NaOH solution gave a very slightly greater increase in amino acids, while the 0.01N H_2SO_4 and the 0.05N Na_2SO_4 solution gave lesser increases. The 0.1N H_2SO_4 and NaOH gave decided decreases during the same period of time.

The sample of Soft Federation wheat had a large increase in amino acids during the first 20 hrs. upon water tempering, then a still larger decrease during the second portion of the tempering period. The 0.01N solutions gave nearly as great increases during the first period, but were constant during the last 72 hrs. The 0.05N Na_2SO_4 tempered sample showed a small decrease during the first 20 hrs., but increased later to about the same figure as the 0.01N NaOH. The 0.1N H_2SO_4 and the 0.1N NaOH caused the amino acid content to decrease exactly the same amount in 20 hrs., slightly more than the Na_2SO_4 sample. By 92 hrs., the samples tempered with the 0.1N H_2SO_4 and the 0.1N NaOH had increased in amino acids to about the same figure as the samples tempered with the 0.01N solutions.

The soft red winter wheat sample, upon water tempering, showed an increase in amino acids during the first 20 hrs., then a very small decrease. When 0.1N NaOH was used instead of distilled water there was an increase in the amino acid and then a falling off during the latter period to about the same value as the water. The 0.01N H_2SO_4 gave only slightly lower results than the distilled water during tempering. The 0.05N Na_2SO_4 solution showed no change during the first 20 hrs., then increased to about the same value as water at 92 hrs. The 0.01N H_2SO_4 and NaOH solutions at 92 hrs. gave about the same results as water upon the amino acid content of this sample.

The hard winter wheat sample behaved similar to the Soft Federation sample when tempered with water, showing a large increase in amino acids during the first 20 hrs., then a still larger decrease during the last 72 hrs. At 20 hrs., all the acid, alkali, and salt solutions lowered the amino-acid content. The 0.01N H_2SO_4 showed the least decrease. The 0.1N H_2SO_4, 0.1N NaOH, 0.05N Na_2SO_4, and 0.01N NaOH showed successive decreases in amino acids. At 92 hrs., the amino acids were about evenly spaced in values, with 0.01N H_2SO_4 at the top, then in decreasing order: 0.1N H_2SO_4, 0.01N NaOH, distilled water, 0.05N Na_2SO_4, and 0.1N NaOH at the bottom.

These results show that the behavior of amino acids upon tempering is a function of the individual sample, rather than the H-ion concentration of the tempering water. But when the results are collected together, certain general differences stand out. Samples tempered with distilled water averaged higher in amino acids than those tempered with the other solutions. The 0.01N NaOH, 0.01N H_2SO_4, 0.1N NaOH, 0.05N Na_2SO_4, and 0.1N H_2SO_4 followed in order of decreasing magnitude in the amino-acid content of wheat when these solutions were used as the tempering agents.

The amino acids apparently follow Loeb's theory of the chemical behavior of proteins, and combine readily with alkalis upon the alkaline side of neutrality, and with acids upon the acid side of neutrality. The results with Na_2SO_4 indicate that the amino acids may combine directly with dissociated salts when the H-ion concentration is close to the isoelectric point of the proteins.

Water-Soluble Nitrogen

At 20 hrs. tempering time, the sample of Hybrid 128 wheat showed a small increase in water-soluble nitrogen when tempered with distilled water. It had the same increase with 0.01N acid and

0.1N acid as with water. The 0.01N NaOH gave a much larger increase than water, and 0.1N NaOH caused double the increase of the 0.01N NaOH. The 0.05N Na_2SO_4 solution lowered the water-soluble nitrogen content.

The water-soluble nitrogen content of the sample of Soft Federation wheat tempered with distilled water increased moderately during the first 20 hrs., and then increased at a slower rate for the remainder of the 92 hrs. All the samples tempered with acid and alkali solutions acted very much alike—each with a greater increase during the first period, and then a decrease at 92 hrs. to about the same value as the water-tempered sample. The sample tempered with 0.05N Na_2SO_4 had a smaller increase during the first 20 hrs., but was equal to the others at the end of the long period.

The sample of soft red winter wheat increased in water-soluble nitrogen when tempered 20 hrs. with distilled water. After 20 hrs there was a falling off to about the original value at 92 hrs. When 0.1N H_2SO_4 was substituted for water, the water-soluble nitrogen content was increased about 0.3% during tempering. The 0.1N NaOH caused a smaller gain than the acid, but there was no decrease in the latter part of the tempering period. The 0.01N acid and alkali at 92 hrs. caused the water-soluble nitrogen content to fall between the water temper and the 0.1N acid temper. The 0.05N Na_2SO_4 when used as the tempering agent gave a lesser increase in water-soluble nitrogen than did water, but the increase continued during the latter part of the tempering period, giving a final result nearly the same as with 0.1N H_2SO_4.

There was a large increase in water-soluble nitrogen content of the hard winter wheat sample when tempered with water, followed by a moderate decrease during the latter part of the tempering period. The sample tempered with 0.1N NaOH had a larger increase during the first 20 hrs., but at 92 hrs. was somewhat lower than the water-tempered sample. The other tempers at 20 hrs. were grouped a little lower than the water-tempered sample. At 92 hrs. all values were closely grouped except the sample tempered with 0.05N Na_2SO_4 solution, which had a much lower water-soluble nitrogen content than the others.

As was the case with the amino acids, the changes in water-soluble nitrogen during tempering are functions of the wheat rather than the H-ion concentration of the tempering water. However, there seems to be a tendency for the 0.1N acid and alkali

to cause a slight increase in the water-soluble nitrogen content of a tempered wheat sample.

All the samples except the Hybrid 128 show a low correlation between the titrable acidity and the water-soluble nitrogen.

Copper Non-precipitable Nitrogen

The copper non-precipitable nitrogen content of the Hybrid 128 wheat decreased slightly upon 20 hrs.' temper with distilled water. When 0.1N acid or alkali was used instead of water, there was an increase rather than a decrease. With 0.01N acid and alkali, and 0.05N Na_2SO_4, the decreases were greater than with water.

The Soft Federation wheat sample increased in copper non-precipitable nitrogen throughout the entire tempering period when tempered with distilled water. The 0.1N NaOH gave slightly higher results at 20 hrs., but the same as water at 92 hrs. When 0.1N H_2SO_4 was substituted for the water, the copper non-precipitable nitrogen was much higher at 20 hrs., but a little lower at 92 hrs. The 0.01N acid and alkali gave somewhat lower results than water. There was a sharp decrease in copper non-precipitable nitrogen when tempered with 0.05N Na_2SO_4 at 20 hrs. After 20 hrs. the copper non-precipitable nitrogen content increased to nearly the original value by 92 hrs.

When the soft red winter wheat was tempered with distilled water, the copper non-precipitable nitrogen first decreased, and then increased at a rapid rate for the remainder of the period of 92 hrs. The use of 0.1N NaOH resulted in an increase instead of a decrease. The 0.1N H_2SO_4 gave an even larger increase in the first 20 hrs. On the other hand, 0.05N Na_2SO_4 caused an even greater decrease than did water. At 92 hrs. the water-tempered sample and the 0.01N samples showed the same large increase in copper non-precipitable nitrogen. The 0.05N Na_2SO_4 sample had nearly as large an increase. The two samples tempered with 0.1N acid and alkali showed much greater increases than the water-tempered sample.

The hard red winter wheat sample showed a large increase in copper non-precipitable nitrogen during the first of the tempering period, and a continued slower increase during the next 72 hrs. All the acid- and alkali-tempered samples acted alike. They gave slightly higher results at 20 hrs., and then were constant for the rest of the time. The 0.05N Na_2SO_4 tempered sample was a little lower than the water-tempered one.

The 0.1N H_2SO_4 and the 0.1N NaOH appear to increase the copper non-precipitable nitrogen when used to temper a sample of wheat. Salt solutions seem to have a decreasing effect. The 0.01N acid and alkali did not vary enough from the water to give consistently different results. The following tables and figures give the results of the above-described experiments.

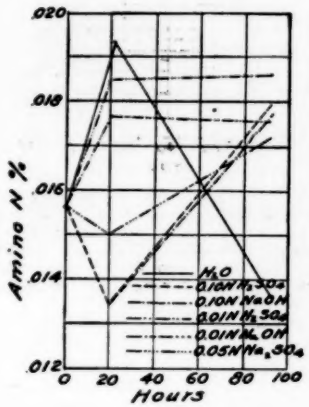

Fig. 1. The effect of varying the hydrogen-ion concentration of the tempering water upon the amino-acid concontent of a sample of Soft Federation Wheat during the tempering period.

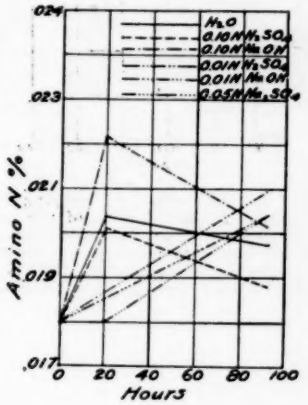

Fig. 2. The effect of varying the hydrogen-ion concentration of the tempering water upon the amino-acid content of a sample of Soft Red Winter Wheat during the tempering period.

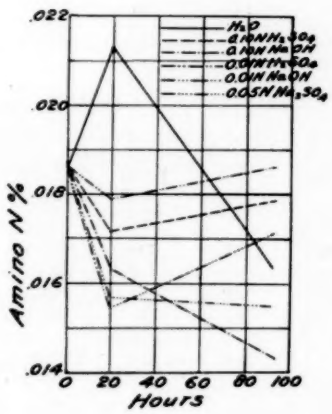

Fig. 3. The effect of varying the hydrogen-ion concentration of the tempering water upon the amino-acid content of a sample of Hard Red Winter Wheat during the tempering period.

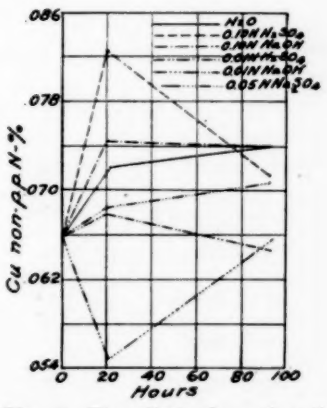

Fig. 4. The effect of varying the hydrogen-ion concentration of the tempering water upon the quantity of nitrogen not precipitable by copper hydroxide in a sample of Soft Federation Wheat during the tempering period.

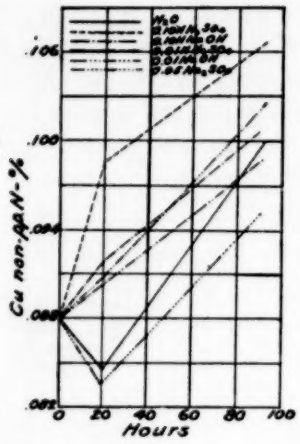

Fig. 5. The effect of varying the hydrogen-ion concentration of the tempering water upon the quantity of nitrogen not precipitable by copper hydroxide in a sample of Soft Red Winter Wheat during the tempering period.

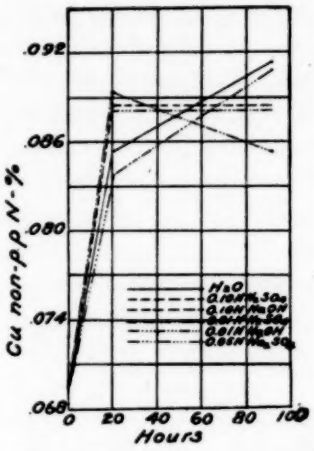

Fig. 6. The effect of varying the hydrogen-ion concentration of the tempering water upon the quantity of nitrogen not precipitable by copper hydroxide in a sample of Hard Red Winter Wheat during the tempering period.

TABLE I

HYBRID 128 WHEAT

Tempered with	Time Temp. Hrs.	Moisture %	Acidity cc. H_2SO_4	Amino N %	Water Sol. N %	Cu. Non-pp. N %
Dry Wheat	0	9.10	2.4	.0124	.254	.0598
Dist. Water	20	17.37	2.35	.0138	.267	.0578
0.1N H_2SO_4	20	16.45	3.3	.0107	.268	.0650
0.1N NaOH	21	16.12	2.35	.0113	.318	.0626
0.01N H_2SO_4	20	17.30	2.10	.0129	.267	.0519
0.01N NaOH	20	16.73	2.15	.0143	.294	.0545
0.05N Na_2SO_4	18	17.67	1.8	.0130	.250	.0494

All results except moisture are calculated to 15% moisture basis.

TABLE II

SOFT FEDERATION WHEAT

Tempered with	Time Temp. Hrs.	Moisture %	Amino N %	Acidity cc. H_2SO_4	Water Sol. N %	Cu Non-pp. N %
Dry Wheat	0	9.30	.0157	2.4	.290	.0659
Dist. Water	21	17.13	.0194	2.25	.306	.0722
" "	92	16.15	.0135	2.55	.314	.0740
0.1N H_2SO_4	20	16.05	.0135	3.35	.325	.0826
" "	92	16.62	.0179	2.85	.317	.0716
0.1N NaOH	20	16.56	.0135	2.25	.320	.0744
" "	92	16.20	.0177	2.05	.320	.0740
0.01N H_2SO_4	20	15.80	.0177	2.7	.324	.0680
" "	92	15.57	.0176	1.9	.302	.0644
0.01N NaOH	20	16.36	.0185	2.65	.321	.0684
" "	92	16.06	.0185	1.95	.313	.0714
0.05N Na_2SO_4	20	16.92	.0150	2.35	.312	.0546
" "	92	16.74	.0172	2.15	.316	.0654

All results except moisture are calculated to 15% moisture basis.

TABLE III

SOFT RED WINTER WHEAT

Tempered with	Time Temp. Hrs.	Moisture %	Acidity cc. H_2SO_4	Amino N %	Water Sol. N %	Cu Non-pp. N %
Dry Wheat	0	10.63	3.4	.0180	.321	.0880
Dist. Water	20	18.18	3.85	.0204	.346	.0846
" "	92	18.88	3.8	.0198	.325	.1000
0.1N H_2SO_4	20	17.65	4.45	.0203	.377	.0984
" "	92	17.75	4.55	.0188	.344	.1070
0.1N NaOH	20	17.17	3.1	.0221	.358	.0920
" "	92	17.27	3.25	.0201	.361	.1008
0.01N H_2SO_4	92	17.90	3.35	.0210	.328	.0987
0.01N NaOH	92	18.06	3.45	.0203	.343	.1024
0.05N Na_2SO_4	20	17.51	4.1	.0180	.336	.0838
" "	92	17.82	3.9	.0203	.345	.0958

All results except moisture are calculated to 15% moisture basis.

TABLE IV

HARD WINTER WHEAT

Tempered with	Temp. Hrs.	Time Moisture %	Acidity cc. H_2SO_4	Amino N %	Water Sol. N %	Cu Non-pp. N %
Dry Wheat	0	10.78	2.45	.0187	.307	.0696
Dist. Water	20	16.06	3.05	.0213	.341	.0852
" "	92	16.54	2.75	.0164	.333	.0912
0.1N H_2SO_4	20	16.58	3.7	.0171	.332	.0886
" "	92	16.58	3.7	.0178	.322	.0886
0.1N NaOH	20	16.49	2.65	.0164	.356	.0886
" "	92	16.41	2.25	.0143	.327	.0886
0.01N H_2SO_4	20	17.00	2.55	.0179	.338	.0892
" "	92	16.36	2.65	.0185	.319	.0852
0.01N NaOH	20	16.17	3.05	.0156	.331	.0884
" "	92	16.21	2.6	.0171	.322	.0884
0.05N Na_2SO_4	20	16.82	2.6	.0157	.332	.0832
" "	92	15.96	3.55	.0156	.304	.0910

All results except moisture are calculated to 15% moisture basis.

Summary

When compared with water used for tempering, 0.1N acid and alkali depress the amino acid content at 20 hrs. At 92 hrs. there are no consistent differences from water. Na_2SO_4 tends to depress the amino acids at all times. The 0.01N acid and alkali have no significant variations from water.

The 0.1N acid and alkali tend to increase the water-soluble nitrogen and the copper non-precipitable nitrogen at 20 hrs., but do not differ materially from water at 92 hrs. Na_2SO_4 is a depressant at all times.

NaOH combines with the proteins more freely than does H_2SO_4. Dilute solutions of NaOH and H_2SO_4, when used as tempering agents, have nearly identical effects upon the nitrogen distribution of the wheats.

EFFECT OF SEVERE WEATHERING ON THE PROTEIN AND ASH CONTENTS OF WHEAT AND FLOUR

W. O. Whitcomb and Arnold H. Johnson
Montana Agricultural Experiment Station
(Read at the Convention, May, 1929)

A preliminary paper entitled, "Effect of severe weathering on certain properties of wheat," was presented by Whitcomb and Johnson (1928) at the Convention in June, 1927. The results reported in the present paper have been obtained by a continuation of this study.

Materials and Methods

The wheats used in this study consisted of Marquis, a hard red spring wheat, and Kanred, a hard red winter wheat. These two wheats were selected because it was desired to compare the effect of weathering on a variety like Kanred, having awns, with that on awnless wheat, like Marquis. Each of these varieties consisted of two lots, one of which was exposed to weathering in the shock, while the other was threshed and exposed to the weather in shallow trays. These trays, which were covered with netting to guard against damage by mice and birds, were supported on frames about three feet above the ground. The object of exposing the threshed wheat in addition to that which was unthreshed was to compare the effect of weathering under these conditions and also insure a more accurate method of obtaining representative samples of the threshed wheat than was possible with that in the shock.

Samplings were made at intervals of about one month for complete tests of the wheats. These samples consisted of one shock of the unthreshed grain and one tray of the threshed grain of each variety. The threshed grain was placed on screens in a room having a temperature of about 25°C. and quickly dried with the aid of a fan. In addition, heads were collected from all the shocks in August, September, and April. These heads were taken from the outside of the shocks, care being taken to select two heads from each bundle. The results of tests on these heads were taken as a good basis of the average test of all the shocks of unthreshed wheat at the beginning of the study. They also served as a measure of the effect of the most severe weathering, since all of the heads were exposed to the elements.

Weathering Conditions

The weathering conditions at Bozeman, Montana, where the study was made, were rather unusual as the fall was open and dry. The snow which came about the middle of December was very heavy and the trays and shocks were soon covered. This snow remained on the wheat without thawing until well into March. The winter temperature was unusually cold, the means of each month being as follows: December, 21.0°; January, 9.9°; February, 14.5°; and March, 31° Fahrenheit. With the exception of a short period during the latter part of December when the temperature rose to 43°F., freezing weather prevailed until March 2nd. The minimum temperature recorded was —36°F.

Results

The results as obtained from protein and ash tests on wheat and flour are given in Table I for Marquis and in Table II for Kanred. In addition to the data for protein and ash contents other data are given which tend to show how the weathering of the wheat progressed. These data are test weight per bushel; dark, hard and vitreous kernels; damaged kernels; weight per kernel; and germination. All of these determinations were made on the wheat which had been dried to 10% or less moisture.

Test weight per bushel showed a greater change as weathering progressed than did any other property of the wheat considered. The unthreshed wheat showed a drop in test weight of 5.2 pounds for Marquis and 4.4 pounds for Kanred. The loss in test weight for the threshed wheat was 5.6 pounds for Marquis and 4.6 for Kanred. It will be noted that Marquis showed a greater loss in weight per bushel than did Kanred, but this cannot be attributed to the awned condition as this same difference existed in both the unthreshed and threshed wheat. A comparison of these changes in test weight with the corresponding weights per kernel indicates that the loss in weight per bushel was not due to any loss of weight per kernel, as this property showed no consistent change from August until April. This would lead to the conclusion that the loss in test weight was due to a decrease in specific gravity, probably caused by a swelling of the kernels when they became wet and their failing to assume normal size when dried out. Specific gravity tests on these wheats showed reductions which corresponded closely with the above mentioned reductions in test weight. This loss in specific gravity for the unthreshed wheat was

0.0673 for Marquis and 0.0357 for Kanred, while for the threshed wheat it was 0.0423 for Marquis and 0.0294 for Kanred. This same condition was observed by Sharp (1926) when he soaked wheat in water and then dried it.

In addition, wheat exposed to the weather showed a decrease in the proportion of dark hard and vitreous kernels, a decrease in germination, and an increase in the percentage of damaged kernels after February 2nd. In the unthreshed wheat the dark kernels decreased from 86% to 17% for Marquis and from 72% to 34% for Kanred. This decrease in the threshed wheat was not quite so pronounced in Marquis, as it was found to be from 80% to 26%. Kanred showed a decrease of dark kernels in the threshed wheat from 70% to 26%. All wheat under test germinated from 98 % to 100% at the beginning of the experiment. No appreciable decrease in this factor was observed until January 2nd, when the threshed wheat of both varieties dropped about 10% in germination. By April 1st the Kanred germinated 70% for the unthreshed wheat and 66% for the threshed wheat, while the Marquis showed 46% and 58% germination respectively.

The grain from the heads which were collected from the outside of the shocks showed more marked changes in the properties just noted than did the wheat in the shocks or in the trays. The increase in damaged kernels was twice as great and the germination dropped to 10% for the Marquis and 23% for the Kanred. These points indicate a much more severe weathering in the outside heads than in either the entire shock or the trays of wheat. It remains to be seen how this difference in degree of weathering was reflected in the protein and ash contents of the wheat.

Changes in Protein and Ash Contents of Wheat and Flour

With these marked changes in the properties of the wheats just noted some changes in the chemical properties might be expected. However, the data on protein and ash, as given in the tables previously referred to, do not bear out this assumption.

The original protein tests in August on the four lots of wheat at the beginning of the experiment showed the Marquis to have a protein content of 10.55% for the unthreshed lot and 10.21% for the threshed lot, while the corresponding data for the Kanred were 9.02% and 9.32%. On April 1st similar tests gave the following results: Marquis, 11.01% protein for the unthreshed and 11.01 for the threshed; Kanred, 9.19% for the unthreshed and 9.49% for the

TABLE I

PROTEIN AND ASH CONTENTS OF WHEAT AND FLOUR AS WEATHERING PROGRESSED — MARQUIS WHEAT

No.	Date Threshed	Test Weight per bu.	Dk. Hd. Vitreous Kernels	Damaged Kernels	Weight per Kernel	Germination	Protein (3)		Ash (3)		Specific Gravity of Wheat (3)
							Wheat	Flour	Wheat	Flour	
		Lbs.	%	%	mg.	%	%	%	%	%	
Wheat in Shocks											
3544	August 17	62.0	86	0	32.4	99.5	10.55	9.75	1.67	.457	1.3767
3546	September 6	62.0	86	0	30.5	99.5	10.41	9.46	1.67	.398	
3548	September 30	61.5	88	0	31.0	99.5	10.45	9.52	1.63	.416	
3552	October 20	61.1	87	0	31.2	100.0	10.50	9.52	1.74	.415	
3650	December 3	60.4	80	0	30.7	99.0	10.21	9.76	1.70	.426	
3705	January 2	58.0	85	0	30.5	97.5	10.90	10.16	1.80	.368	
3881	February 2	58.1	43	0	29.8	78.5	10.90	10.06	1.72	.371	
4064	March 1	57.2	14	1.2	31.0	85.5	10.55	9.81	1.75	.374	
4096	April 1	56.8	17	12.2	31.8	46.0	11.07	9.91	1.74	.412	1.3091
Wheat in Trays											
3550	Original (1)	61.8	80	0	31.4	100.0	10.21	9.37	1.68	.382	1.3793
3651	December 3(2)	58.5	64	0	31.9	97.5	10.01	8.38	1.67	.400	
3706	January 2	58.5	51	0	31.8	87.5	10.50	10.33	1.68	.381	
3882	February 2	57.5	45	0	31.6	85.0	10.35	9.62	1.68	.387	
4065	March 1	56.7	24	6.0	30.9	57.0	10.35	9.66	1.79	.429	
4097	April 1	56.2	26	11.3	31.6	58.5	11.01	9.91	1.70	.439	1.3370
Heads collected from shocks.											
1	August 30		62	0	32.5	97.5	11.30		1.63		
3	September 20		50	0	32.3	99.5	11.31		1.64		
5	April 1		19	21.0	32.2	10.5	11.31		1.70		

(1) Original wheat as threshed before being exposed to weather. Trays of this wheat were placed on frames in the field Oct. 29, 1929.
(2) Dates on which the threshed wheat was brought in from the field where it was exposed to weather.
(3) Basis 13.5% Moisture.

TABLE II

PROTEIN AND ASH CONTENTS OF WHEAT AND FLOUR AS WEATHERING PROGRESSED — KANRED WHEAT

No.	Date Threshed	Test Weight per bu.	Dk. Hd. Vitreous Kernels	Damaged Kernels	Weight per Kernel	Germination	Protein [1]		Ash [3]		Specific Gravity of Wheat [3]
							Wheat	Flour	Wheat	Flour	
		Lbs.	%	%	mg.	%	%	%	%	%	
Wheat in Shocks											
3545	August 17	63.1	72	0	37.3	99.5	9.02	8.48	1.59	.361	1.3792
3547	September 6	61.0	66	0	36.1	99.5	8.73	7.94	1.55	.346	
3549	September 30	60.6	68	0	36.4	100.0	9.17	8.43	1.58	.342	
3553	October 20	61.3	65	0	37.3	100.0	9.17	8.33	1.63	.384	
3652	December 3	60.2	57	0	37.0	99.0	9.17	8.48	1.74	.359	
3707	January 2	59.0	58	0	36.3	99.0	9.02	8.33	1.73	.343	
3883	February 2	57.7	36	0	37.6	93.5	9.12	8.43	1.73	.335	
4066	March 1	57.1	17	3.0	36.7	69.5	9.12	8.73	1.64	.354	
4098	April 1	58.7	34	1.3	37.4	70.5	9.19	8.43	1.63	.388	1.3435
Wheat in Trays											
3551	Original [1]	62.0	70	0	37.3	98.5	9.32	8.38	1.68	.360	1.3850
3653	December 3[2]	60.2	50	0	36.9	93.0	9.42	8.48	1.63	.381	
3708	January 2	58.3	35	0	37.2	84.5	9.12	8.48	1.62	.348	
3884	February 2	57.5	26	0	36.5	59.5	8.97	8.63	1.65	.363	
4067	March 1	56.7	17	1.0	36.5	44.0	8.82	8.58	1.71	.402	
4099	April 1	57.4	26	6.0	36.9	66.5	9.49	8.63	1.67	.378	1.3556
Heads collected from shocks											
2	August 30		26	0	37.6	98.5	9.50		1.56		
4	September 20		33	0	37.5	99.0	9.37		1.56		
6	April 1		16	12.0	36.5	23.0	9.84		1.57		

(1) Original wheat as threshed before being exposed to weather. Trays of this wheat were placed on frames in the field Oct. 29, 1929.
(2) Dates on which the threshed wheat was brought in from the field where it was exposed to weather.
(3) Basis 13.5% moisture.

threshed. These results of protein tests on wheat were confirmed by the corresponding tests on the flours milled from these wheats. These results, together with the fact that the protein content was fairly regular according to the tests made from month to month, would seem to indicate that the change which took place in protein content was not significant even though the changes in the physical properties and the germination of the wheat were marked.

In August the ash contents of the wheats were as follows: Marquis, unthreshed lot 1.67%, threshed lot 1.68%; Kanred, 1.59% and 1.68% respectively. The tests in April showed that these ash contents had remained practically constant during the winter. The April ash tests were as follows: Marquis, 1.74% for unthreshed and 1.70% for threshed; Kanred, 1.63% and 1.67% respectively. As in the case of the protein tests, these results of the ash tests were confirmed throughout the season by ash tests on the flours from these wheats.

The protein and ash contents of the wheat from the heads which were selected from the outside of the shocks remained as constant as did these constituents from the wheats from the entire shocks and the trays, even though the weathering was more pronounced. The initial protein content of the Marquis was 11.30% and that on April 1st was 11.31%. The corresponding tests on Kanred showed 9.50% and 9.84% respectively. The ash contents of these two wheats remained just as constant as did the protein contents, the initial test for Marquis being 1.63% and the April test 1.70%, while for Kanred they were 1.56% and 1.57% respectively.

A comparison of the effect of severe weathering on the 1928 crop of wheat as herein reported with that of the 1926 crop, as reported in 1927, shows certain interesting features. The same reduction in test weight per bushel without any significant change in weight per kernel was observed in both cases. Similar reductions in dark kernels and germination with an increase in damaged kernels were found in both cases. Likewise, the protein content of the wheat remained rather constant for both crops, with the exception of the initial drop of about 2% in the 1926 crop which might be attributed to lack of uniformity in samples. The ash content of the wheat remained comparatively constant for both years.

The baking and chemical tests which are being made on these wheats and flours will furnish additional data of interest. The results of this phase of the work are not available at present.

Conclusion

The protein and ash contents of wheat which was subjected to severe weathering conditions were not changed even though marked changes were observed in the physical properties and in germination.

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SUNDRY MEANS OF HASTENING THE DETERMINATION OF PROTEIN OF WHEAT

HERBERT L. WILKINS [1]
Kansas State Grain Inspection Department
Kansas City, Mo.
(Read at the Convention, May, 1929)

PART I.

Apparatus and Present Procedure

The introduction of protein content as a factor in determining the price of wheat has brought with it an insistent demand for unprecedented speed in making that determination. For the most part in former times the analyst could make the protein determination along with his other work at his own convenience. This procedure occasioned little inconvenience or loss to his employer, because the wheat so examined was usually already purchased and, in any case, the protein content was then seldom a factor in the price. At present, however, the delay of a few minutes may cause serious loss if the price changes or if the market closes before the report is received.

Consequently, the State Laboratories, as well as others, have done everything possible to hasten the work without impairing its accuracy.

As chief chemist for the Kansas State Protein Laboratories the author has had the opportunity of conducting investigations in this field. It is the purpose of this paper to present and discuss the results of these investigations. Very great savings in time and la-

[1] Read by Mr. Ralph Culver.

bor were effected by routines and apparatus devised during the winter of 1926-1927.

The manipulations involved in the conduct of an analysis must of course consume a large share of the total time. The first reduction of this time resulted from the use of permanently numbered sample envelopes, thus eliminating the practice of writing identification material on these envelopes with the consequent necessity of erasing it before again using the container. The present equip-

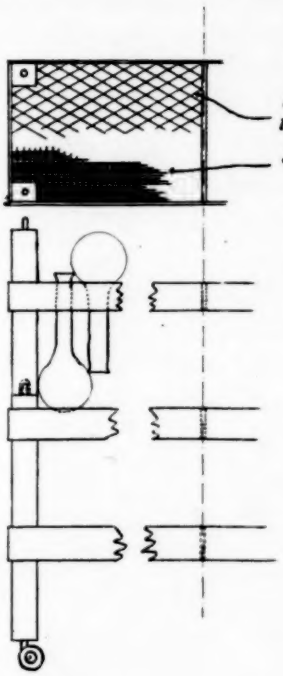

Fig. 1. Interchangeable Flask Rack and Construction of Floor Under Carts

ment comprises 3,200 of these envelopes. Similarly, the cans which contain ground material were numbered permanently and for the same reason. There are 2,200 of these. They are stored in consecutive order in drawers which hold 100 cans each. Since almost a negligible amount of time is required to keep them in order the saving in time is apparent when it is remembered that formerly when there were as many as three or four hundred samples daily it required three or four people about two hours each day to erase and re-number the cans and to erase the envelopes.

Racks for use at the balances and washing sink, and for drying and storing the flasks, are so made that they will hold about 90 flasks each and will fit interchangeably upon each other or on

the carts. These make for ease of handling and economy of floor space, since (See Fig. 1.) 1,200 of the 800 cc. flasks can be accommodated on 14 racks. Two of these may be kept under the digester on the two short-legged carts, two others will always be needed at the balances, and a third usually stands at the washing sink on one of the long-legged carts. The remaining flasks then require only two sections of floor space, each of which is 2 ft. wide and 4 ft. long.

The use of an electric coffee mill will greatly hasten the grinding operation since it does not heat or clog with wet samples, and cleans itself between samples. (See Fig. 2.)

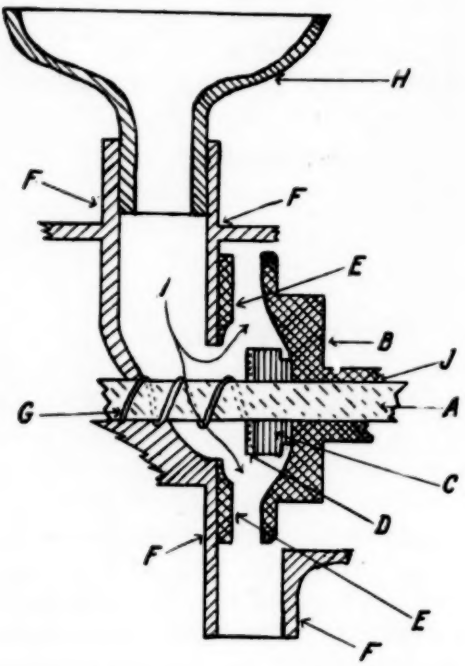

Fig. 2. Sample Grinder

A—Extension of the motor shaft.
B—Rotating burr assembly.
C—Laminated cylinder made up of pressed fiber washers and faced with an iron washer D, and rotated with B because of the pressure of G against D.
D—Iron washer facing for C.
E—Stationary burr.
F—Partial section of grinder-head casting.
G—Coil spring to prevent B from striking E while running idle.
H—Section of aluminum hopper.
I—Course of grain from H through the burrs.
J—The clearance between A and B is sufficient to allow B to slip back and forth on A easily.

The cooling racks are so designed that one whole digester full of flasks (30, all faced the same way) may be quickly cooled by the aid of an electric fan, diluted, and supplied with anti-bump and alkali without handling. This is an obvious advantage. (See Fig. 3.)

Dilution is made with a garden hose which is fitted with a plunger cock, to which an anti-splash screen is attached.

The tanks (Fig. 4, A) in which the strong alkali is prepared and stored are made from steel barrels (2 ft. in diameter) so cut

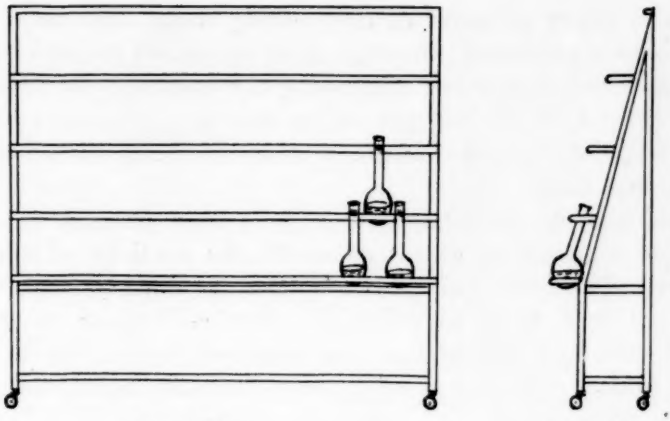

Fig. 3. Cooling Racks

in two that they are 2 ft. high. They are mounted on platforms provided with strong casters. Seventy-six liters of water (including a solution of 7¾ pounds of $Na_2S_2O_3$) are put into the tank. To this is added (all at once) a 125 pound drum of flake alkali, and stirring with a suitable paddle is begun immediately. After about 10 min. solution will be complete.

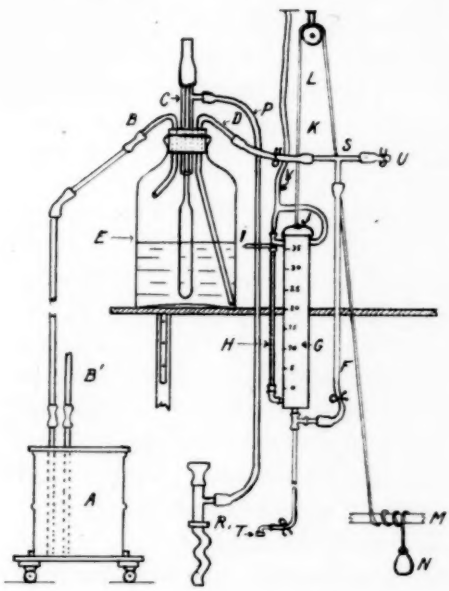

Fig. 4. Apparatus for Dispensing Concentrated Alkali

A—One of the steel tanks.
B—Glass and rubber tubing conveying alkali from A to E.
C—Automatic shut-off device. (See Fig. 5.)
D—Siphon through S and F to G.
E—Five gallon bottle.
F—Rubber hose connecting both five-gallon bottles to G thru S.
G—Concentrated alkali dispensing burette.
H—Graduated glass gauge.
I—Pointer by which to set G for filling.
J—Bail by which the burette assembly is suspended.
K—Window sash cord.
L—Pulley attached to ceiling.
M—Iron pipe or rod.
N—Weight attached to the loose end of K.
O—Ten-foot rubber delivery tube.
P—Rubber hose from the five-gallon bottle to the suction pump.
R—Suction pump (Richards).
S—Glass T tube in siphon from E to G.
T—Ordinary "anti-splash" strainer often used on faucets at sinks.
U—Branch of siphon leading to the other five gallon bottle.
V—Branched breather tube leading to the gauge tube and to the upper end of the burette.

The strong alkali reagent is dispensed from a 2½ liter steel burette (Fig. 4, G), through a delivery tube (Fig. 4, O) long

enough to reach all parts of the cooling rack. This tube is furnished with a pinchcock, although most operators prefer to use the thumb and first finger for controlling the outflow. An anti-splash screen (Fig. 4, T) on the end of the delivery tube serves to produce a large, slow stream of alkali which does not stir up the contents of the flask.

This burette is fitted with a glass tube (Fig. 4, H) which serves as a gauge by means of which the portions of alkali are measured. The two 5 gal. service bottles are filled by suction from the 40 gal. steel tanks in which the solution is made up. Each of these bottles is fitted with a long-stemmed float (Fig. 4, C; and Fig. 5) which stops the flow of alkali into the bottle by opening

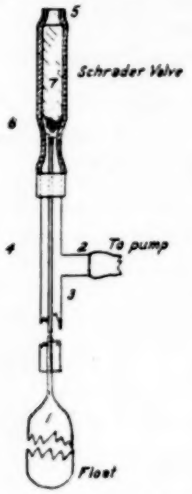

Fig. 5. Float for Alkali Dispensing Apparatus
1. Float.
2. Side-neck.
3. T-tube, guide for 4 and support for 7.
4. Long stem of 1.
5. Sectional view of 7 and 6.
6. Large-headed box nail.
7. Schrader bicycle valve.

a Schrader valve when the bottle is full. Hence they may be left to fill without attention.

If the carboy of concentrated H_2SO_4 can be kept in the laboratory, this system will be a great help when applied to the handling of that reagent. In addition to the use of the above indicated system for handling the acid (or independently of it) a burette (Fig. 6, K) for dispensing the acid is so made that it is filled by a self-stopping siphon (Fig. 2, H) from the supply container. Thus all pouring of acid from one open vessel to another is avoided (and time is saved as well) since the siphon works without attention while the operator is placing his flasks on the digester.

The receivers (12 oz. wide-mouthed bottles) are permanently numbered, using the same code as is used on the cans and envelopes, except that there are only 500 pairs of official sample receiv-

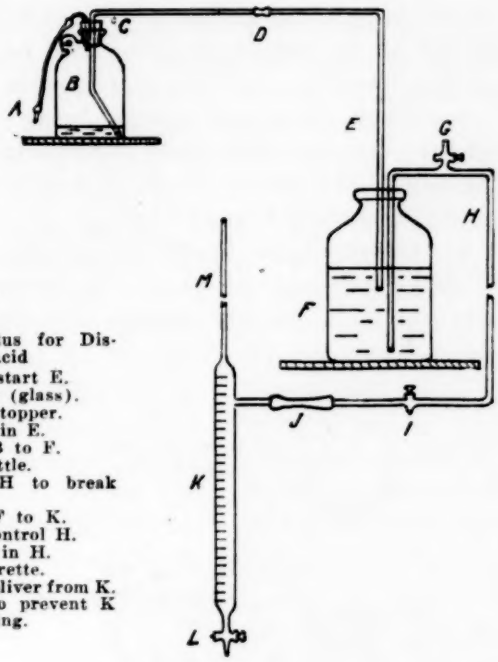

Fig. 6. Apparatus for Dispensing Sulfuric Acid

A—Blow-tube to start E.
B—One-gallon jug (glass).
C—No. 8 rubber stopper.
D—Flexible joint in E.
E—Siphon from B to F.
F—Five-gallon bottle.
G—Stopcock in H to break the siphon.
H—Siphon from F to K.
I—Stopcock to control H.
J—Flexible joint in H.
K—Dispensing burette.
L—Stopcock to deliver from K.
M—Safety tube to prevent K from overflowing.

ers and 50 pairs of submitted sample receivers. Again the system of handling them in trays containing ten numbers (20 bottles) and emptying an entire tray full of bottles at once by means of a dumping frame requires no time for keeping them in order and avoids about three or four operations which were formerly necessary. (See Fig. 7.)

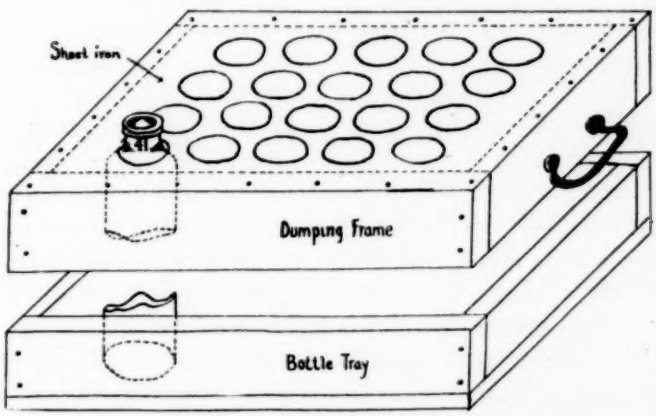

Fig. 7. Trays for Receiving Flasks

Another feature which the author presumes is not generally used is the addition of the indicator to the acid before it is dispensed. This acid solution is made of approximate strength and used without adjustment, the proper volume being determined by titration against the standard alkali using the standard burette.

Weighing is done at the rate of about 120 to 150 weighings per hour per man. Dry flasks are always used.

An important feature in the distillation process is the use of Kjeldahl traps which have large by-pass holes in the tubes below the stoppers (See Fig. 8). This arrangement prevents all splatter-

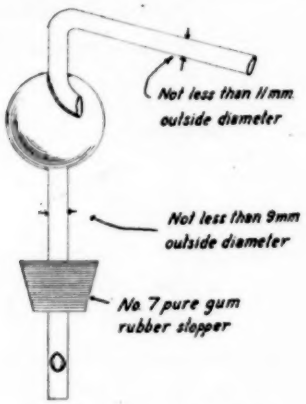

Fig. 8. Connecting Bulbs

ing in the bulbs. The Pyrex bulbs should be furnished with tubing having an outside diameter of not less than 11 mm. on the upper end and not less than 9 mm. on the lower end. It is advisable to use pure gum-rubber stoppers since they are soft enough to make installation easy and do not shrink as much with use as do ordinary ones.

Titration of the receivers is done with calibrated Schellbach automatic burettes marked from the bottom upwards and provided with three-way glass stopcocks. The use of eighth normal (0.12524 normal) NaOH allows the percentage of protein to be read directly from such burettes. In the busy season the percentages thus read are called off to a clerk who enters the values on the record cards, calculates the average, and enters it on the pan ticket. Two titrators can keep a good clerk working at top speed.

The laboratory is equipped with 120 stills and 120 digesters.

After the rush season in 1927, the writer decided to use the winter of 1927-28 to make a study of the various phases of the di-

gestion and distillation problem, and also to further perfect the apparatus and routine. Happily the work of O. M. Shedd (1927) came to the author's notice through Chemical Abstracts, soon after the publication of the original article. This work provided both a starting place and spur to activity. The first study consisted of 50 determinations which were made on our regular daily control sample using the 20 min. digestion period recommended by Shedd. These values agreed well with those obtained in the daily routine. Next the factor of safety in this period was determined by making similar tests using various digestion periods ranging from 10 to 35 minutes. Fifty determinations were made in each of these groups also. These experiments showed that the 10 min. period had digested over 97% of the protein present in the sample. Hence there is a generous margin of safety in the 20 min. digestion period when the official method of the American Association of Cereal Chemists is used with heat equivalent to that produced by 600 watt Gilmer heaters.

Many investigators have reported that the conventional 150 cc. given in most procedures is more distillate than is necessary. However, many of them did not see fit to recommend any change in the official method and as far as the author knows there is at present no change in any of the official procedures with respect to this matter. The minimum adequate volume of distillate was determined by receiving six different volumes of distillate (50-65-80-95-110-125 cc.) from flasks into which a solution of an ammonium salt had been pipetted and to which the regular amounts of other reagents except the digestion powder had been added. Thirty determinations were made in each of the six groups. A large majority of the values in the 65 cc. group were good, and all succeeding groups contained a normal number of good values; hence it was considered that 95 cc. provided a sufficient margin of safety. (Since these experiments were performed in a similar manner to those reported in Part II, they are not detailed here.)

This is briefly the basis for our present method which uses the above outlined short-cuts in manipulation, a 20 min. digestion period on previously heated heaters, and 95 cc. of distillate which can be boiled over in about 20 min. In all other respects the method is that of the American Association of Cereal Chemists. An experienced operator having hot heaters ready can make a duplicate determination on one sample in this laboratory in 45 min.

On at least one occasion we were able to finish 728 samples in duplicate in 11½ hours with a crew of thirteen men. Later in the season, with a smaller, more experienced crew, (six men, one on part time) we were able to operate at a rate of 20 determinations (10 samples) per hour per man, that is, 265 samples (530 determinations, in 4½ hours.

This rate of operation, however, is not fast enough. For example, there were several days during the summer of 1928 when there were from 700 to 1,000 samples; obviously, there is a definite need for a still speedier process in order to be able to finish the work during the peak movement without excessive overtime.

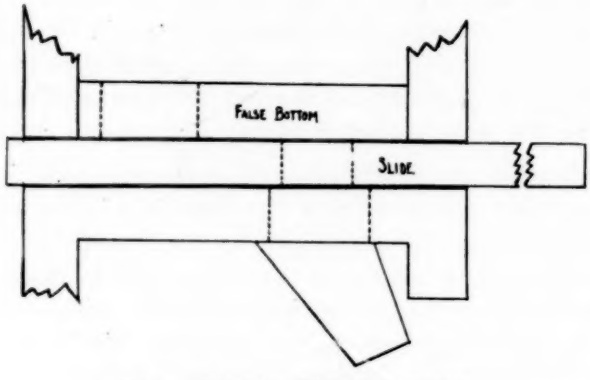

Fig. 9. Digestion Mixture Dispenser.

The efforts of the author to meet this situation are presented in Part II of this paper.

Since this paper was first prepared, the author has installed a device for dispensing the digestion mixture. It is much the same in principle as an old fashioned hand-operated corn planter. The essential features are diagrammed in Figure 9.

Summary of Part I

The influence of protein content on the price of milling wheat, the volume, and seasonal character of the crop movement have virtually forced the inspection laboratories to use all possible speed in their work.

In this discussion it has been shown that the process is hastened greatly by the use of procedures and devices which enhance either the speed, accuracy, or convenience of the operation.

Most of these require little or no attention from the operator. Notable among these are the means of

1. Grinding and storing the sample
2. Dispensing the powder
3. Dispensing strong acid
4. Dispensing strong alkali
5. Cooling the flasks after the digestion
6. Diluting the digest
7. Handling the receivers to and from the storage shelves, stills, and titration table
8. Emptying a tray full of bottles at once
9. Identification of sample containers
10. Identification of distillate receivers
11. Preparation of reagents in bulk
12. Reading the percentages directly from the burette
13. Simultaneously dispensing the receiver acid and the indicator.

Relieving the titrators of the necessity of stopping to make records of the readings, and the convenience of the anti-bump used in the distillation process (⅜ inch glazed porcelain marbles) also make for greater speed.

The methods by which the shortened periods of digestion and distillation were established are outlined in Part I and are exemplified in Part II of this discussion.

The maximum operating rate of 10 samples (20 determinations) per man per hour was obtained by the use of the procedure and equipment described. A maximum output of 1,600 determinations representing the completion of the analysis of 728 samples was accomplished in an 11½ hour day.

PART II.

A Fast, Reliable Modification of the A. A. C. C. Method for the Determination of Protein

The first effort toward a faster procedure was a preliminary survey of the effect of varying amounts of powder and of catalyst in the powder. Table I is a summary of this work. While there is not, in most cases, enough data to be conclusive, there is evidence enough to be indicative of the probable results of a more thorough investigation. A study of the table shows that excessive amounts of catalyst appear to have no detrimental effect, that the use of even 1 gm. of HgO to each 100 gms. of K_2SO_4 produces a very

TABLE I

A — Effect of Varying Amounts of the Digestion Mixture

Number of determinations	Minutes digested	Grams of powder used	Gms. HgO added to 100 gms. K_2SO_4	Ave. deviation of protein percentages from average %	Normal val. of Cont. No. 7. (ave. nearest 4 days)	Ave. % protein for each group	Deviation of aves. from normal value
30	15	7.5	6	±0.060	12.29	12.01	—0.28
30	15	10.0	6	±0.052	12.16	12.11	—0.05
47	15	15.0	6	±0.049	12.15	12.09	—0.06
30	..	23.0	6	(Digestion was so slow that the determinations were not finished.)			
		B — Effect of Using Longer Digestions, Other Factors Same as Above					
30	20	7.5	6	±0.053	12.22	12.01	—0.21
30	20	10.0	6	±0.050	12.29	12.20	—0.09
		C — Effect of Varying the Amount of HgO in the Digestion Mixture					
15	15	11.5	0	(very large)	12.29	11.70	—0.59
6	15	12.5	1	.....	12.40	11.97	—0.43
6	15	12.5	2	.....	12.40	12.18	—0.22
6	15	12.5	3	.....	12.40	12.31	—0.09
6	15	12.5	4	.....	12.40	12.23	—0.17
6	15	12.5	5	.....	12.40	12.29	—0.11
15	15	11.5	16	±0.050	12.29	12.20	—0.09

marked improvement, and that amounts greater than 3 gms. per 100 appear not to be of any further benefit. Hence it was concluded that our regular mix (6 parts HgO:100 parts K_2SO_4) was reasonably near the optimum for a routine process. Again the results of this study and of at least one other recent investigation indicate that somewhat more than the usual ten grams of powder is nearer the optimum than is the smaller amount. Our usual 11-13 gm. dispenser is not then greatly in error. In the trial using excessive amounts of powder (23 gms.) the digestion proceeded so slowly that the determinations were not completed. In the case of the trial in which 16 gms. of mercuric oxide were mixed with 100 gms. of K_2SO_4, a proportionately increased amount of the NaOH-$Na_2S_2O_3$ solution was used to precipitate the mercury.

Next it was planned to conduct some experiments using hotter heaters. Accordingly a local manufacturer of domestic heating appliances was consulted. He advised shortening the present coils and was kind enough to calculate the amount of shortening which would be necessary to produce an 800 watt element. Several of these were prepared and digestions and distillations made upon them. These trials indicated that a very material reduction was possible in the time required for both digestion and distillation. However, the necessity of using a heater that would produce one-third more heat is, in our case, very objectionable since the temperature in the vicinity of the digestors is very uncomfortable in the summer time with the present heaters. It is feared that it would be unbearable if the hotter heaters were used. These considerations led the author to the line of investigation which has resulted in the method which this paper proposes.

From the standpoint of theory at least, one equivalent of increasing the amount of heat on the digestor would be to decrease the amount of material to be heated. Some digestion trials were made using one-half as much sample and reagents as are ordinarily used. These tests were so promising that a few 500 cc. Kjeldahl flasks were purchased. Asbestos plates were made in which the holes were of such size that the smaller flasks were held at the same distance above the heaters as the regular ones when the plates were laid on top of the regular cap. These preliminary tests indicated many apparent advantages such as a digestion period at least as short as could be used with the 800 watt heater, the sides of the flasks were flushed down by the condensate much more quickly than in the case of any previous trials, and it was surmised

that the amount of distillate necessary to be received from the smaller flasks would be less, since the ratio of the total volumes of the two flasks is 67 to 100.

Experiments were planned and carried out to settle the above questions and also to establish the reliability of the 0.5 gm. sample as compared to the usual 1 gm. sample in the case of both homogeneous and non-homogeneous materials.

For use in these experiments reagents of half strength (except the strong acid and alkali, of which half portions were used) were prepared by the usual methods. The standard acid is HCl prepared by the constant boiling method. It is dispensed into the titration vessel with a Bureau of Standards pipette and is titrated with the unknown alkali from a Bureau of Standards burette using the regular amount of Alizarine red as indicator. A special dipper was made with which to dispense the digestion powder. (6 parts HgO mixed with 100 parts of K_2SO_4.) This dipper delivered from 5.7 to 6.1 grams of powder.

For these digestions 600 watt heaters were used. One pair was equipped with regular Gilmer Caps for 500 cc. flasks and the other pair was equipped with the asbestos plates already mentioned. Both 600 and 800 watt heaters were used in the distillation trials.

No marked advantage from the standpoint of recovery of ammonia was noted in any of the trials comparing the yield from the two rates of distillation. Possibly more carefully designed and executed experiments would show what, if any, advantage accrues from increasing the rate of distillation aside from the obvious one of economy of time.

The initial experiment to determine the necessary volume of distillate failed because of the use of tap water to dilute the charge, and perhaps also because of the ill-advised use of NH_4Cl solution as the source of ammonia. In repeating the work, an $(NH_4)_2SO_4$ solution (5 grams made up to 1 liter) was used as the source of ammonia, and 125 cc. of distilled water, 10 cc. of commercial concentrated H_2SO_4, and 30 cc. of strong alkali (gravity 1.5 NaOH—$Na_2S_2O_3$ solution) were added to each flask. The charge of water and acid was measured into the receivers and the amount of distillate determined by comparison with a similar bottle containing the proper volume of water. A 25 cc. transfer pipette (Bureau of Standards) was used to dispense the $(NH_4)_2 SO_4$ solution into the flasks. The calculated reading upon our direct reading burettes is 15.11%, assuming absolute purity of the $(NH_4)_2SO_4$.

Table II is the record of the results of this experiment.

It is of interest to note that there is a range of only 0.021% in the averages of the last four groups in the small flask—distilled water test. Also even 80 cc. of distillate from the 800 cc. flasks did not produce an average nearly as high as did 40 cc. from the small flasks.

TABLE II

VOLUME OF DISTILLATE STUDIES

A — 500 cc flasks diluted with 125 cc. of distilled water.

	20 cc. dist.	40 cc. dist.	60 cc. dist.	80 cc. dist.	100 cc. dist.
	14.45	15.05	15.05	15.05	15.05
	14.10	15.05	15.00	15.05	15.05
	14.55	15.05	15.05	15.05	15.00
	14.60	15.05	15.00	15.05	15.05
	14.55*	15.05*	15.00*	15.05*	15.05*
	14.45*	15.00*	15.00*	15.05*	15.05*
	14.85	14.95	15.05	15.10	
	14.80	15.05	15.00	15.00	
	14.85	15.05	15.05	15.05	
	14.85	15.05	15.05	15.05	
	14.95*	15.00*	15.05*	15.05*	
	14.95*	15.05*	15.00*	15.00*	
Averages	14.662	15.033	15.025	15.046	15.041
Ranges	0.85	0.10	0.05	0.10	0.05

B — 800 cc. flasks diluted with 150 cc. of distilled water.

	14.35	14.80	14.90	15.05
	14.40	14.95	14.90	14.95
	14.80	14.85	14.75	15.05
	14.85	14.90	14.90	15.00
	13.95	15.00	14.95	14.90
	14.25	15.00	14.95	14.85
		15.00		
		14.85		
		14.90		
		14.90		
		14.95		
		14.95		
Averages	14.443	14.920	14.891	14.966
Ranges	0.90	0.15	0.20	0.20

C — 500 cc. flasks diluted with 125 cc. of tap water.

	15.00	15.05
	15.00	15.00
	15.00	15.00
	15.05	14.95
	15.00	14.95
	15.00	14.95
Averages	15.01(—)	14.983
Ranges	0.05	0.10

* 600 watt heaters used. For all others 800 watt heaters were used.

Since the minimum adequate volumes of distillate from the two flasks is approximately proportional to their total capacities, it seems logical that the minimum volume of distillate is a function of the space to be freed of ammonia. The fact that nearly all the ammonia is passed over with only 20 cc. of distillate also supports this conclusion.

The results of these and other tests using tap water serve only to indicate that more work must be done on that phase of the problem. Considering the results of these distillation tests it is seen that 75 cc. of distillate is nearly 1.9 times the absolute minimum.

The sample upon which the time of digestion studies were made was the January check sample of the Kansas City Section of the American Association of Cereal Chemists. The results which were reported upon this sample were unusually concordant. A generous supply of this sample was obtained through the courtesy of Mr. Edgar Karbach of the Missouri State Protein Laboratory, who prepared and distributed the January check for the Section. Table III is a copy of his report upon this check (omitting the chemists' names).

TABLE III

(Report on the January Check Sample of the Kansas City Section of the American Association of Cereal Chemists)

Collaborator		Determinations ("as received" basis)		
		Moisture	Protein	Ash
1		....	12.44	....
2		11.86	12.53	1.846
3		11.63	12.41	1.855
4		11.50	12.55	1.862
5		11.65	12.41	1.858
6		11.40	12.30	1.860
7		11.92	12.51	1.821
8		11.70	12.40	1.800
9		11.38	12.47	1.882
10		11.56	12.50	1.850
11		11.58	12.55	1.880
12		11.60	12.42	1.835
14		11.72	12.36	1.856
15		11.50	12.55	1.820
16		.....	12.50	.....
	Average	11.62	12.46	1.848
	Maximum	11.92	12.55	1.882
	Minimum	11.38	12.30	1.800
	Range	0.54	0.25	0.082

Our preliminary digestion experiments showed that some good results could be obtained with a 6 min. digestion period. The digest was nearly clear in about 4¼ min. on the average. It was decided therefore that a digestion period of 12 min. would be taken as the

TABLE IV

DIGESTION STUDIES CONDUCTED BY THREE ASSISTANTS, USING THE CHECK SAMPLE REPORTED IN TABLE III

Protein values found	Digested 6 Minutes				Digested 12 Minutes				Digested 18 Minutes			
	No. of each by Mr. "A"	No. of each by Mr. "B"	No. of each by Mr. "C"	Total cases each value	No. of each by Mr. "A"	No. of each by Mr. "B"	No. of each by Mr. "C"	Total cases each value	No. of each by Mr. "A"	No. of each by Mr. "B"	No. of each by Mr. "C"	Total cases each value
11.95			1	1								
12.00			1	1								
12.05			2	2								
12.10	1			1								
12.15				0								
12.20			1	1								
12.25			2	2								
12.30	1	1		2								
12.35	2	1	5	8								
12.40	4	3		7	1			1		2	1	3
12.45		2	3	5			1	1	2			2
12.50	4	2	3	9	2	4	1	7	6	4	2	12
12.55		2	1	3	3	3	9	15	8	3	8	19
12.60	4	2	1	7	12	13	6	31	3	8	5	16
12.65		3		3	2		3	5		4	3	7
12.70		3		3				0		1		1
12.75				0								
12.80		1		1								
	Total det'ns 6 min.			56	Total det'ns 12 min.			60	Total det'ns 18 min.			60
	Average protein			12.437%	Average protein			12.574%	Average protein			12.556%
	Avge. deviation:				Avge. deviation:				Avge. deviation:			
	From 12.45			±0.137%	From 12.60			±0.034%	From 12.55			±0.048%
					From 12.55			±0.044%				

tentative routine time. To establish the validity of this decision each of the three assistants in the Kansas City Laboratory was directed to make 20 determinations (upon the sample described above) using three different digestion periods, namely, 6, 12, and 18 minutes. The amount of distillate received was 75 cc., and the method of determining that volume was the same as was used in the distillation studies. The flasks were diluted with 150 cc. of tap water. Table IV presents the data concerning this experiment.

A study of Table IV shows that 65% of the results in the 6 min. group were within the range of the other two groups, that is, between 12.40% and 12.70%. Also it may be seen that there is a very minute advantage in the 12 min. period over the 18 min. one with respect to range, average per cent of protein, and average deviation from the reading nearest the mathematical average.

It is obvious that the selection of 12 min. as an adequate digestion period is justified with respect to homogeneous samples. There remains to be determined the question of the effect of lack of uniformity. In order to investigate one phase of this problem one hundred samples (mostly official but including also a few appeal samples) were repeated the same or the following day, by the short method. These results are shown in Table V, in which

TABLE V

A Study of the Reliability of the Half-Portion Method upon Routine Samples

Range of differences (above and below the reference value) included in each group.	500 re-checks* vs. their original values, both being derived from analyses upon the original grinding using the regular method.		100 determinations by the half-portion method vs. the values obtained by the regular method. Both analyses made upon the original grinding.		4,000 duplicate determinations made during the rush season in 1928. The comparison shows the difference between the two values.	
	Percentage of values falling in each group.	Running totals of the previous column.	Percentage of values falling in each group.	Running totals of the previous column.	Percentage of values falling in each group.	Running totals of the previous column.
Exact checks	12.2	12.2	13.0	13.0	16.85	16.85
0.01 — 0.05	22.4	34.6	19.0	32.0	21.78	38.63
0.06 — 0.10	20.4	55.0	28.0	60.0	21.73	60.36
0.11 — 0.15	16.8	71.8	20.0	80.0	14.93	75.29
0.016 - 0.20	11.8	83.6	11.0	91.0	11.35	86.64
0.21 — 0.25	6.0	89.6	6.0	97.0	5.13	91.77
0.26 — 0.30	1.8	91.4	0.0	97.0	5.20	96.97
0.31 — 0.35	3.2	94.6	3.0	100.0	3.03**	100.00
0.36 — 0.40	2.6	97.2				
0.41 — 0.45	0.8	98.0				
0.46 — 0.50	0.8	98.0				
0.51 — 0.55	0.4	99.2				
0.56 — 0.60	0.4	99.6				
0.61 — 0.65	0.4	100.0				

* 508 re-checks were surveyed in order to get the 500 reported above. The 8 discarded samples varied from the original by more than 0.65% and for this reason it was considered that some factor aside from the process was operating.

** This value (3.03%) also includes all differences greater than 0.35%.

the performance of the 500 most recent rechecks, and the agreement between duplicate determinations in the case of 4,000 samples (from the files of July and August, 1928) is used as a basis for comparison.

In general the results are as might have been expected. That is, the short method makes a better showing than do the rechecks. It compares much better, however, with the agreement between duplicates. It may be concluded from this study that the short method is just as reliable as the regular one when applied to ordinary samples.

Next, four samples were prepared by mixing wheats of very different protein contents.

Sample "A" was made up from unknown amounts of six samples which contained the following percentages of protein at the time of mixing—14.25, 15.00, 15.00, 10.25, 11.13, and 9.85%. These discarded samples were dumped into a pan and passed once through the Boerner Sampler. Six successive grindings were made without further effort at mixing the sample before grinding. Four determinations were made upon each of the six grindings by both methods.

Sample "B" was composited from two samples one of which had 16.30% of protein and the other 10.38%. Three parts of the low were used with two parts of the high and then mixed just as was Sample "A." Seven successive grindings were made from this composite, the computed value of which is 12.75%. Four determinations were made upon each grinding by both methods one day, and similarly the following day duplicate determinations were made upon the same grindings.

Samples "C" and "D" were also composited from two wheats. The low one was a sample of white wheat. The high one was the very highest quality hard winter wheat. The respective protein contents of the two samples at the time of mixing was 16.08% and 10.10%. The grinding and mixing of samples "C" and "D" was done exactly as in the case of sample "B."

Sample "C" was made from three parts of the low protein wheat and one part of the high. The calculated value of this composite is 11.60%. The analyses were made by the regular method one day and by the short method the next day.

Sample "D" contained three parts of the high protein wheat and one part of the low. The calculated value is 14.58%. Otherwise samples "C" and "D" were treated alike.

Table VI is a condensed summary of the work upon these samples.

TABLE VI

THE RELIABILITY OF THE HALF-PORTION METHOD UPON NON-HOMOGENEOUS SAMPLES

Sample Number	Average protein by the regular method.	Average protein by the half-portion method.	Average deviation of the values by each method from the computed value of the respective composites.		Number of determinations on each sample by each method.
			Regular Method	Half-portion Method	
A	12.37	12.55	(not a composite.)		24
B	12.68	12.69	±0.224	±0.260	42
C	11.65	11.63	±0.197	±0.201	14
D	14.57	14.52	±0.213	±0.181	14

Again, no real difference in the performance of the two methods is revealed in this study which, while not exhaustive, is at least indicative of the status of the two methods with respect to non-homogeneous samples. That this conclusion applies to the three types of sample is shown by summarizing the three previous experiments in Table VII.

A few examples are presented which show the minimum time in which an acceptable value can be obtained by an experienced operator, using the shortest digestion period which the heaters (600 watt) will allow, 0.5 gm. samples, appropriate amounts and concentrations of reagents, 40 cc. of distillate with 800 watt heaters on the stills, and the time-saving devices previously described.

One assistant in the Kansas City laboratory obtained some good values in 16 min. from the time he sat down to the balance until the titrations were made. An assistant in one of the other laboratories similarly obtained some good values in 15 min. Full time can be allowed for all operations and one sample may be determined in 25 min.

Summary of Part II

This discussion has shown the possibility of greatly shortening the time required to complete the determination of protein in wheat by using (1) 0.5 gm. samples in 500 cc. flasks, (2) 12 min. digestion on previously heated 600 watt Gilmer heaters, (3) 75 cc. of distillate, (4) appropriate amounts and concentrations of reagents, and (5) the apparatus discussed in Part I.

The minimum adequate volume of distillate has been determined for the stated amounts of reagents when used in a 500 cc. Kjeldahl flask. From this data, this volume appears to be a func-

TABLE VII

A SUMMARY OF THE VARIOUS COMPARISONS OF THE HALF-PORTION METHOD WITH THE REGULAR ONE

Description of the several reference standards, and the material compared with them.	HOMOGENEOUS SAMPLES		ROUTINE SAMPLES			NON-HOMOGENEOUS SAMPLES		
	Average of 60 determinations (12 min. group, table 4)	Average of 60 determinations (18 min. group, table 4)	Original official analyses (500) on samples rechecked (reg. meth.) See table 5	Original official analyses (100) on samples re-determined (by half-meth.) See table 5	Low one of the duplicate determinations (4,000) See table 5	Average of 40 values, by reg. method on 4 samples vs. same data by half-portion meth. See table 6	Computed value of the composite, (28 comparisons.) vs. 94 det'ns by reg. method See table 6	Computed value of the composite, (28 comparisons) vs. 94 det'ns by half-portion method. See table 6
Avge. dev. from std.	±0.034	±0.048	±0.127	±0.106	—0.109	±0.131	±0.214	±0.225
% of detns. above std.	8.5%	40.0%	56.6%	58.0%	.....	62.5%	50.0%	50.0%
Avge. amt. above std.	0.050	0.069	0.159	0.127	.....	0.148	0.185	0.195
% of detns. exact chks.	51.7	31.7	12.2	13.0	16.85	2.5	3.5	0.0
% of detns. below std.	40.0	28.3	31.2	29.0	.....	35.0	46.5	50.0
Avge. amt. below std.	0.075	0.074	0.118	0.110	.....	0.112	0.261	0.257
% above	8.3	40.0	56.6	58.0	.....	62.5	50.0	50.0
% below + % chks	91.7	60.0	43.4	42.0	.....	37.5	50.0	50.0
No. detns. each group	60	60	2000	400	8000	188	94	94

tion of the space from which the ammonia must be removed and is independent of the rate of distillation.

The digestion period is dependent on the amount of total material in the digest, on the ratio of the reagents to each other and on the nature of the sample but not on the per cent of protein in the sample.

The use of the 0.5 gm. sample is shown to be just as reliable as is the 1 gm. sample.

That there is an adequate margin of safety in the proposed method is evidenced by the data submitted and by the fact that some acceptable results may be obtained in 3/5 of the proposed time.

Approximately 575 determinations (not duplicates) were made especially for the use in this paper and in addition approximately 9,000 determinations upon official samples were reviewed in compiling this data.

Acknowledgments

In the course of a series of investigations, such as have been reported in these papers, it is inevitable that the author should have received aid from a great many sources.

First, his instructors, employers and associates in the Kansas State Agricultural College, in The New Mexico College of Agriculture and Mechanical Arts, and in Purdue University, laid the foundation for the conduct of this work.

Finally, his employer, assistants and associates in the Kansas State Grain Inspection Department have provided the means of giving substance to his ideas.

To all of these the author wishes to express his sincere thanks.

Literature Cited

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1927 Rapid boiling as an aid to a shortened period of digestion in the determination of nitrogen. J. Assoc. Offic. Agr. Chem. **10**:507-20.

THE DETERMINATION OF ASH BY THE DIRECT WEIGHT METHOD

E. O. KLOPFSTEIN

Red Star Milling Co., Wichita, Kansas

In the fall of 1925 the author, under the pressure of daily increasing numbers of ash tests, began to compute ash by weighing directly. The initial experiments proved so satisfactory that in January, 1926, the method was adopted for all routine work. The method was found to be easily adapted to flours from both spring and winter wheat. All routine ashes for each succeeding crop have been made by direct weight. In each case it has proven reliable.

Table I has been compiled to give the author's results on collaborative samples sent out by the Pioneer Section of the A.A.C.C. in comparison with the results of two other laboratories chosen from among the largest mills in the section. One of these chemists used the direct weight method and the other used the official indirect method. The table covers a period of thirteen samples. The average result of all the collaborators is considered correct and equivalent to 100%. For the purpose of comparison each milligram is considered 1%, so that a variation of one milligram from the collaborative average would make the score 99%, two milligrams 98%, and so on. The average score of the collaborator running ashes by the official method was 93.2%. The collaborator determining ash by the direct weight method scored 94.8%, and the author scored 95.3%. Liddel[1] (1929) of the Department of Agriculture says, after extensive investigation of the different ways for determining ash: "Not only did the direct ash weights check those made in crucibles, but on the whole were more uniform." This is a convincing answer to the objection that ashes weighed directly absorb moisture rapidly; and it is to be noted that he took care to observe and compare the absorption of hygroscopic moisture in all methods.

In our ordinary routine work we have found the following procedure satisfactory. Five grams of the sample are quickly weighed into an aluminum scoop. The sample is then transferred to the crucible. The crucibles are then placed in an electric muffle whose temperature is 593° C. (1100° F.). The door of the muffle is left open. When the flame has died out the furnace is allowed to cool to 420° C. (800° F.) with the door remaining open. The door is closed and the rheostat adjusted so that the temperature will rise

[1] Cereal Chem. 6: 134

slowly and steadily to 579° C. (1075° F.) and allowed to remain at this point until three hours have elapsed from the time the samples were put in. Then the heat is increased until the temperature is 593° C. (1100° F.). After an hour at this heat the ashes are sufficiently burned. Usually the time required for burning is approximately four hours.

The crucibles are removed to a desiccator and allowed to cool until they are just warm to the touch. Then the crucible is taken out and the ash transferred to a counterpoised watch-glass on the balance pan. If small particles of ash cling to the sides of the crucible they may easily be removed with the point of a spatula and dumped out upon the watch-glass.

The most satisfactory crucible which we have used is that made of Vitreosil of 15 cc. capacity, having a diameter of 41 mm. and height of 25 mm. We have used a few Sillmanite crucibles. About once each week we scour the inside of the crucibles with fine emery cloth. This polishes the inner surface of the crucible and prevents the adherence of ash particles. Each day we wipe the crucibles out thoroughly with a soft cloth.

TABLE I

COMPARISON OF AVERAGE RESULTS OF ASH DETERMINATION MADE BY PIONEER SECTION OF A. A. C. C WITH THE AUTHOR'S DETERMINATION BY DIRECT WEIGHING AND TWO COLLABORATORS, ONE DIRECT WEIGHING AND THE OTHER WEIGHING IN CRUCIBLE.

	Average of Collaborating Laboratories	Author's Determination, Direct Weighing	One of Collaborators, Direct Weighing	One of Collaborators, Weighing in Crucible
	Per Cent	Per Cent	Per Cent	Per Cent
	0.477	0.476	0.496	0.470
	.407	.406	.410	.400
	.377	.374	.376	.390
	.615	.611	.620	.615
	.397	.394	.392	.400
	.413	.418	.416	.425
	.368	.360	....	....
	.429	.420	.436	.440
	.397	.390	.394	.395
	.397	.389	.392	.395
	.413	.410	.406	.400
	.474	.480	.476	.465
	.483	.480	.486	.480
Average	100%	95.3%	94.8%	93.2%

TABLE II

ASH RESULTS USING DIFFERENT TIME AND TEMPERATURE

	566° C 8 Hours	599° C 4 Hours
	Per Cent	Per Cent
	0.372	0.371
	.474	.480
	.654	.654

TABLE III

THE ASH DETERMINATIONS WERE MADE ON FIVE GRADES OF FLOUR BY DIRECT WEIGHING AND BY WEIGHING IN CRUCIBLE. THE AVERAGE TIME TO WEIGH THE ASH BY WEIGHING IN CRUCIBLE WAS 90 SECONDS AS AGAINST 54 SECONDS BY DIRECT WEIGHING OF THE ASH.

Weighing in Crucible Grams	Direct Grams
0.0187	0.0185
.0220	.0220
.0243	.0240
.0335	.0332
.0357	.0355

TABLE IV

COLLABORATIVE SAMPLES WERE USED. THE ASH DETERMINATIONS WERE MADE FIRST BY WEIGHING IN THE CRUCIBLE. AFTERWARDS THE ASH WAS TRANSFERRED TO THE BALANCE PAN AND WEIGHED DIRECTLY. PLATINUM CRUCIBLES WERE USED AND TIME OF BURNING WAS 16 HOURS.

Average of Collaborating Laboratories	Author's Determination using Platinum Crucibles	
	Weighing in Crucible	Direct Weighing
Per Cent	Per Cent	Per Cent
0.498	0.503	0.504
.436	.440	.437
.415	.423	.420
.530	.520	.522
.447	.453	.453
.650	.654	.650
.417	.422	.416
.453	.460	.450
.890	.890	.890
.398	.389	.392
100%	94.2%	96.4%

We have found 593° C. (1100° F.) the optimum temperature for our furnace and equipment. It is quite probable, however, that men in other laboratories will find somewhat different time and temperature for burning more suitable to their apparatus. Coleman and Christie[2] (1925) observed that fushion began at 585° C. (1085° F.). Notwithstanding this, we have burned large numbers of ashes at 593° C. (1100° F.) and if fusion occurred it did not affect the results materially. In fact each of three samples were burned for four hours at 593° C. (1100° F.) and eight hours at 565° C. (1050° F.). (See Table II.) In each instance the variations were negligible, easily falling within that margin allowed for experimental error.

The author has now used this method of weighing ash directly since 1925 and it is his opinion that if each individual will endeavor to adapt the process to his own equipment it will, by virtue of its simplicity, prove a boon to any cereal chemist.

The author wishes to thank Franklyn Edwards of our own laboratory for assisting him in composing this paper.

[2] Cereal Chem. 2: 291.

BOOK REVIEWS

Bread. A collection of popular papers on wheat, flour, and bread. By Harry Snyder. With a biographical sketch by Andrew L. Winton. 293 pages. Price $2.50. The Macmillan Company, New York. 1930.

As the sub-title indicates, this is a collection of selected papers written by the late Harry Snyder during the past thirty years. These papers had been previously published in a variety of journals and magazines, and cover quite a diversity of subjects. The technical papers are prefaced by a short biographical sketch prepared by Dr. Andrew L. Winton, and a short appreciation of Prof. Snyder's work in the field of soils chemistry, prepared by Dr. F. J. Alway.

The book includes chapters bearing the following titles:

Introductory, William C. Edgar
Biographical Sketch, Andrew L. Winton
Prof. Harry Snyder as Soils Investigator and Teacher, Frederick J. Alway
Prefatory Note
Food Habits and Flour Consumption
White Flour, a Wholesome Food
The Digestibility of Breads
The Relative Value of White Flour and Whole Wheat Flour
Mineral Components of Wheat and Flour and Their Role in Human Nutrition
Camouflage Bread
The Bread-Fed Man the Well-Fed Man
Improved Methods of Flour Milling
The Old-Fashioned Flour Mill
The Grading and Merchandising of Wheat Flour
The Flour Moisture Standard
The Mill Chemist
Some of the Flour Miller's Problems
The Milling Value of Wheat
A Working Knowledge of Flours
The Granulation of Flour
Modern Milling of Hard Wheats
Some of the Baker's Problems
The Individuality of Wheat
What Science does for Farm Crops
Soil Wealth and Its Conservation
Wheat in Times of War and Peace
Washington as a Wheat Farmer
Writings of Harry Snyder
Index

The book is of value not alone for the technical material which it contains, but also because it constitutes a record of the varied pioneer work engaged in by this leader in the field of cereal chemistry. Naturally the views of chemists have changed somewhat during the past thirty years, and all who read this document may not be in entire agreement with certain of the technical conclusions recorded in some of the earlier papers. It is quite significant, however, that in the main the observations of this ingenious worker have been largely supported by subsequent developments, and his findings exerted a large influence in the development of the chemical technology of the milling industry and in guiding the preparation of definitions and standards utilized in the governmental control of the quality of flour and bread.

All cereal chemists and technologists who have pride in the development of their field of specialization will find a place for this document on their bookshelves, and will make extensive use of it as a record of the evolution of numerous phases of cereal chemistry.

—C. H. Bailey.

"Cerco"—Proceedings of the First International Conference on flour and bread manufacture, in Prague, September 22, 1927. Edited by Francis Hruška, 578 pages. Published by "Cerco," Prague I.—606, Staroměstské 16. 1929.

To anyone who is interested in the milling and baking industries, this report should prove both interesting and helpful. In addition to a complete report of the meetings, each of the 49 contributed papers is given in full, in the language in which it was presented. There were four official languages at the conferences: Czech, English, French and German. In preparing this report, Dr. Hruška has included detailed abstracts of each article, in each of the official languages, thereby adding much to the usefulness of the report.

The contributed papers included the following:

J. Žák: Milling and baking value of the Czechoslovakian wheats.
J. Mička: Comparison of American and European milling industry with a short description of different flour produced in America.
V. Kočka: Silo "Suka" with the aeration of the chambers.
Ch. Branberger: Effect of the art of milling and grade of wheat flour on its quality.
Ch. Křtinský: Improved flour milling after preceding chemical peeling of the grain.
S. Camilla: Uniform type of flour in Italy and advantages resulting from the peeling of the berry in moistened condition.
M. Arpin: The requirements of millers for the quality of grain.
M. T. Pécaud: French State Milling School.
M. M. Pakuto: Use of electrical energy for the purification of middlings.
Fr. Haluška: Requirements of the Bakers concerning the quality of flour.
J. Mertl: Why the Czechoslovakian baker is still buying foreign flour.
D. W. Kent-Jones: Improving baking quality by physical means.
O. Toužimský: Improving of the grain and flour in the physical way by means of a special apparatus.
W. Brabender: The history of flour maturing by means of air nitrogen and its compounds produced in electrical way with respect to newest situation of the technique.
H. K. Parker: An industrial application of nitrogen trichloride.
O. Czadek: Methods of flour maturing.
Kurt Ritter: Flour improving by chemical means.
W. Kranz: The Humphries Process.
G. van der Lee: A) Flour improving by means of the process "Novadel.' B) Flour improving by means of the preparation "Multaglut."
J. Kulman: Correlation of the baking value of rye flour to the optimal quantity of the leavening gas in the manufacture of the pumpernickel.
B. Svoboda: Technical and economical disciplines in the baking shop.
M. Arpin: Essays on flour.
M. T. Pécaud: Baking tests.
F. E. Thomas: The ideal yeast food.
J. R. Chittick: Baking powders, their composition and application.
L. Elion: Improving dough fermentation by addition of yeast food.
Ferd. Jirásek: The requirements of consumers for the pastry quality.
St. Růžička: Criticism of all manipulations carried out with grain and flour from the standpoint of national health and national nutrition.
J. Stoklasa: Modern opinions on the nourishment of the human and animal organism.
W. Glaser: The patented process of Steinmetz from the standpoint of human nutrition and national economy.
V. Žemlička: The modern manufacture of margarine and its significance in the baking shop.
O. Svozil: Effect of the addition of vegetable oil on pastry.
F. L. Dunlap: Good bread.
Kurt Ritter: The significance of flour improving by chemical means in relation to the food value of our bread nutrition.
F. Hruška: Effects of artificial flour improving on the food value of pastry and on health.

M. Arpin: Methods for control of the milling process and the milling products.
G. van der Lee: New laboratory apparatus for the determination of the dough consistence and bread volume.
J. Jelínek: Apparatus for the determination of hardness of the kernel.
Josef Adorján: Rapid water determination in milling laboratories.
J. Koblic: Sand in flour with respect to the future Czechoslovakian food codex, and to the desirable international settlement of this question.
B. Sucharda: A new method for the microscopy of flour.
J. Koblic: Determination of the grade of flour by determining its ash content.
C. G. Harrel: Testing and use of flour in bread production.
J. Koblic: Determination of the grade of flour by determining its fat content.
K. Křtinský: Determination of the degree of flour extraction on the ground of its chemical analysis.
C. H. Bailey: The role of hydrogen ions in bread production.
A. H. Johnson: Factors influencing the viscosity of flour-water suspensions.
R. C. Sherwood: Diastatic activity and flour milling.
R. Urban: Importance of the popularization of knowledge on grain, flour and bread.

At the proposal of Dr. Arnold H. Johnson, who was the official representative of the American Association of Cereal Chemists, it was adopted that "The Methods of the A. A. C. C. as published in 1928, will be used as a basis for working out the International Methods." It is hoped that by the standardization of methods, there will be less opportunity for disputes arising over the analyses of cereal products.

The reviewer understands that this document will be sold for about $3.50.

—J. A. Dunn.